THE NATIONWIDE CASE-COHORT STUDY IN PHARMACOEPIDEMIOLOGY: A STUDY OF IATROGENIC ANAPHYLAXIS AND AGRANULOCYTOSIS

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THE NATIONWIDE CASE-COHORT STUDY IN PHARMACOEPIDEMIOLOGY: A STUDY OF IATROGENIC ANAPHYLAXIS AND AGRANULOCYTOSIS

De landelijke case-cohort studie in de farmaco-epidemiologie: een studie van iatrogene anafylaxie en agranulocytose

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Voor Gommert
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Part I

Introduction and scope

1

Introduction to drug safety

They who make it their profession to sell or prescribe drugs carry a shared responsibility for the monitoring of drug safety (Yearly report, Netherlands Centre for Monitoring of Adverse Reactions to Drugs, 1993).

1.1 What is drug safety?

Drug safety is an important part of postmarketing surveillance. In ancient times, people were already aware of the fact that drugs could have side effects. The oldest drugs were mainly of plant and animal origin, but also mercury, arsenic and antimony were used, the toxic effects of which were well known. In 1224 Frederick II, Emperor of Hohenstaufen ordered inspection of drugs and mixtures prepared by apothecaries and in 1518 the Royal college of Physicians was founded, whose Fellows were also concerned with quality control of drugs. The first drug to be banned because of its toxicity was antimony in the 17th century. It was used again, however, after it cured Louis XIV from typhoid fever.

One of the first descriptions of an intoxication to a drug which is still in use today, was made by William Withering in 1785. He described digitalis intoxication as follows: "The Foxglove, when given in very large and quickly repeated doses, occasions sickness, vomiting, purging, giddiness, confused vision, objects appearing green or yellow, increased secretion of urine with frequent motions to part with it, and sometimes inability to retain it; slow pulse, even as low as 35 in a minute, cold sweats, convulsions, syncope and death" [1].

In the same era, calomel (mercurous chloride), often mixed with other purgatives, was used to treat yellow fever. Many patients survived this treatment, perhaps aided by the vomiting they already did. Others developed mercurialism with intense salivation, loosening of the teeth, ulceration (sometimes even gangrene) of mouth and cheeks, and osteomyelitis of the mandible. In the 19th century, however, calomel was used as a treatment for all febrile diseases. Some laymen understood the danger of calomel, and after a while physicians started to add to their protests. Holmes wrote in 1861: "if the whole materia medica, as it is now used, could be sunk to the bottom of the sea, it would be all the better for mankind - and all the worse for the fishes".

In the 19th century, the first pharmacopoeias were made, and at the end of this century and the beginning of the 20th the first formal enquiries into suspected adverse reactions to drugs were made: sudden death during chloroform anaesthesia, and jaundice after arsenical treatment of syphilis. Not until the end of the 1930's, a Federal Act was introduced in the U.S.A. which forbade the marketing of new drugs until they had been cleared for safety by

the Food and Drug Administration. In The Netherlands, however, such legislation was implemented as late as the early fifties.

In 1961, it was discovered that the popular hypnotic thalidomide was an important teratogenic agent. This led to the institution of the Netherlands Centre for Monitoring of Adverse Reactions to Drugs in 1963, as well as to the establishment of other drug safety offices worldwide. Later, the World Health Organization (WHO) set up an international bureau to collect data on adverse reactions to drugs from all over the world. Today, this system is still in use: a computer link to Sweden (Uppsala) connects all drug safety offices in Europe and gives them access to a large databank. All countries linked to the system also have to feed the databank with their data on adverse reactions to drugs. Every year, a meeting is organized by the WHO in order to discuss drug safety items that have come up during the past year. Currently, the European Community plans to implement a similar network.

Aided by the number of law suits to pharmaceutical companies and the often exaggerated presentation by the media, drug safety has received much attention during the past 30 years. Pharmaceutical firms take great care in order to ensure that all possible adverse reactions will be mentioned in their product information sheets, in order to prevent law suits obliging the payment of large sums of money to the victims of suspected adverse reactions to their drug. However, the field of drug safety has mainly focused on voluntary reporting in the past decades, and systems and methods for postmarketing surveillance have not been fully developed yet. There are some special problems involved when studying adverse reactions to drugs.

As mentioned, drug safety is an important part of postmarketing surveillance. Postmarketing surveillance, however, encompasses more than drug safety. According to the Dutch Health Council, postmarketing surveillance is systematic surveillance of and scientific research into all desired and undesired (side) effects of drugs on health, as soon as these drugs are marketed. Therefore, postmarketing surveillance should yield data needed for rational and safe use of drugs, but, strictly speaking, also of medical devices. It not only encompasses the area of research into side effects of drugs, but also the research of efficacy and effectiveness of drugs [2]. Although the definition formally pertains to drugs used by humans, also veterinary drugs are subject to postmarketing surveillance.

The safety and efficacy of a drug are investigated in depth during the premarketing phase. After production and selection of a chemical entity with therapeutic potential, the pharmacological and toxicological effects of new drugs are first studied in animals. The LD 50 for several animal species are assessed (i.e. the dose at which 50 % of animals die due to the toxic effect of the drug), and the effect of the drug on animals is investigated. Then, Phase I to III studies are performed, in which drugs are first tested on healthy

volunteers and later on patients with the disease against which the drug is supposed to be effective. However, by the time a drug is marketed, clinical experience is still limited.

This thesis will discuss the pharmacoepidemiologic approach to drug safety in the postmarketing phase. Pharmacoepidemiology studies the frequency, distribution and determinants of diseases in populations, with the drug as one of the determinants. Safety is often described in terms of adverse effects or adverse reactions, side effects or adverse events. Often, the meaning of these terms is misunderstood. Hence, the definitions of these terms are given below:

- An "adverse effect" or "adverse drug reaction" is defined by the World Health Organization as "one which is noxious and unintended, and which occurs at doses used in man for prophylaxis, diagnosis or therapy". The important part missing from this definition, however, is the causal relationship. A drug should of course have been used within a proper time window before the event (challenge), a reversible event should subside after discontinuation (dechallenge), and if the drug is readministered, a similar reaction would be expected (rechallenge).

- A "side effect" is every unintended effect caused by a drug used in therapeutic doses. This effect can be wanted as well as adverse, as long as it is unintended and caused by the drug. Depending on the aim of the therapy, a pharmacological effect can be the intended therapeutic effect of the drug or a side effect. For example, morphinomimetics can in addition to their analgesic effect also cause constipation and are therefore sometimes used to treat diarrhoea.

- An "adverse event" is every noxious and unintended effect occurring during or after use of a drug in therapeutic doses. Such events are not necessarily caused by the drug.

- An intoxication is a noxious effect caused by a drug used in higher than therapeutic doses, irrespective of the intention of the individual who took the overdose.

In short, there are three groups of adverse effects:
1. pharmacological adverse reactions
2. allergic adverse reactions
3. other idiosyncratic adverse reactions

1. Pharmacological adverse reactions are effects that are explained by the pharmacology of a drug. These effects are therefore predictable.

2. Allergic reactions are immunologically mediated reactions, and their occurrence depends on the vulnerability of the patient who is using the drug. These reactions are therefore unpredictable.
3. Examples of other idiosyncratic reactions, often on the basis of a metabolic aberration or deficiency, are haemolytic anemia caused by certain drugs in patients with glucose-6-phosphate dehydrogenase deficiency or peripheral neuropathy due to isoniazid in slow acetylators. These reactions are also unpredictable unless the status of a patient regarding such types of metabolism or deficiencies is known.

1.2 Why is relatively little known about drug safety before marketing?

Before a drug is registered, there are four stages of research. First, the drug is selected and animal experiments are performed in order to study its pharmacological activity and toxicity. Subsequently, phase I clinical trials are performed: healthy volunteers are studied in order to examine the metabolism and the pharmacological activity of the drug. Thereafter, phase II clinical trials are performed, in which the clinical effect of the drug is studied in small groups of selected patients. Then phase III clinical trials are performed on large groups of selected patients. These are mostly prospective, randomized, controlled, and double blind. After these studies, a preliminary benefit-risk assessment is made.

In these premarketing studies, the pharmacological adverse effects are usually found. The problem, however, is that the drug is usually
- only tested on 1000-2000 people (at the most)
- not tested on elderly people, children and pregnant women
- tested for relatively short periods of use
- tested on people who have only the disease which can be treated with the drug and are otherwise without major illnesses
- tested while people are not using other drugs.

In fact, the real experiment will therefore take place in phase IV, the postmarketing phase, in which the drug is used for the first time on a large scale and under everyday circumstances. Not until this phase will rare adverse effects be found (such as allergic and idiosyncratic reactions) and will more be learnt about interactions with other drugs and about the safety of using the drug in non-tested groups, such as pregnant women. Not until this phase can the value of a drug be assessed during widespread use under everyday circumstances, and can a new balance of benefit and risk be made.

The aim of postmarketing surveillance is:
- to discover unknown side effects/adverse effects (qualitative aspect)

- to measure the incidence of and risk factors for side effects/adverse effects (quantitative aspect)
- to reconsider the benefit/risk ratio of the drug and to compare it to that of related drugs
- to study diagnostic tools for, and mechanisms and prevention of adverse effects.

1.3. Systems and methods for postmarketing surveillance in The Netherlands

There are several systems and methods for postmarketing surveillance in The Netherlands. A system is defined as an accessible and ordered data set on the level of individual patients, with regards to prescriptions, morbidity, or a combination of both. Such systems may vary from drug exposure databases or disease registries to a registry encompassing only reported suspected adverse reactions. Some of these systems facilitate record linkage. Record linkage refers to the linking of morbidity and exposure data in two different databases, e.g. on the basis of an individual's social security number. Examples of record linkage systems are the Saskatchewan Health Plan in Canada and COMPASS in the U.S.A. Both consist of databases which were primarily implemented for registration of billing data of health professionals, so called health maintenance organisations or other types of health insurance funds. In this way, however, they contain data on prescriptions and morbidity. A problem with this type of databases is that they were not designed with the intention to perform postmarketing surveillance studies with their data. As a result, these databases do not contain information which may be relevant to the outcome of interest (such as smoking status). Moreover, some diagnoses are better paid by the health maintenance organisations than others, which creates a bias towards the registration of such diagnoses. A specific problem of the Medicaid database is that it concerns a health care facility for the poor, and therefore is not representative of the total population. For a more extensive review of the large record linkage systems, the reader is referred to Strom [3].

A postmarketing surveillance method pertains to the way in which the relationship between a drug and a disease is studied. Here, we can make use of case-reports or case-series when we focus on the clinical description of adverse reactions. When studying the frequency of and risk factors for adverse reactions according to pharmacoepidemiologic standards, a control group is important. This can be achieved by performing cohort studies, case-control studies, or cross-sectional studies. These are the principal methods used in epidemiology and pharmacoepidemiology. In the following sections a more extensive description is given of the most commonly used systems for

postmarketing surveillance in The Netherlands, and of the methods which are used to this purpose.

1.3.1 Postmarketing surveillance systems in The Netherlands

There are several data collections concerning drug use and morbidity in The Netherlands, but only some are useful for pharmacoepidemiologic studies. A short description of some important systems is given below.

1.3.1.1. Reporting systems

1. Netherlands Centre for Monitoring of Adverse Reactions to Drugs

Since 1963, this centre is located at the Ministry of Health, nowadays in Rijswijk. Its monitoring system works with voluntary reporting. Suspected adverse reactions to drugs are directly reported by physicians and pharmacists, or via pharmaceutical companies.

If a suspected adverse reaction is reported, the likelihood of a causal relationship between the adverse event and the drug is examined. Reporting physicians are often contacted by telephone and asked for more detailed information. Also, in the literature and in the registry itself it is checked whether there are more reports of this adverse event on the same drug. If so, a causal relationship might be more likely. When adverse reactions are reported to one central agency, it is easier to collect a case series of the same adverse reaction to a particular drug. Such series often lead to a publication in a medical journal or, if necessary, to further research. Since case reports are published in medical journals, there is feedback of knowledge about adverse reactions to other health professionals. By cooperating with centres for monitoring of adverse reactions to drugs from different countries and by pooling such data at the World Health Organisation Collaborating Centre for International Drug Monitoring much information is available.

The centre has recently transferred its reporting scheme to the Netherlands Pharmacovigilance Foundation, and changed its name to Drug Safety Unit.

2. The Netherlands Pharmacovigilance Foundation (LAREB)

The Netherlands Pharmacovigilance Foundation (LAREB) started as a regional reporting system in Tilburg in 1984, with the idea that if reporting adverse reactions to drugs would be done more frequently, more signals would be received, and more information would be obtained from the system. Also, the

regionalisation would more easily facilitate feedback to general practitioners about drugs and drug use and could contribute to prescription policies within the region. Since 1991, the regional registry has expanded to nine regions in The Netherlands, with a national coordinating centre. The aim of the foundation is to cover the whole country in 12 regions. Local pharmacists collect the information from physicians and patients and in the regional centre this is classified as to the probability of the adverse event being an adverse reaction to the drug. After evaluation, the final report is fed into the central database [4, 5]. The communication lines between reporter and LAREB may be quite long, since a physician reports to a pharmacist, who in turn reports it to the regional REB, from where it goes into the LAREB databank. It is, however, also possible to report directly to the LAREB. The advantage of the system is, that the reporting pharmacist is able to add the prescription history of the patient to the report. Since January 1996, the LAREB foundation is designated as holder of the national voluntary reporting system in The Netherlands.

Advantages of voluntary reporting systems are:

1. They are quick, flexible and simple
2. They are cost-effective
3. The whole population of users of a drug can be followed during the total period of registration of a drug
4. The voluntary reporting system selects patients who are liable to develop idiosyncratic reactions, and acts as a starting point for further research

Disadvantages of voluntary reporting systems are:

1. Under-reporting:
   - adverse reactions which are well-known are seldomly reported
   - unknown adverse reactions are not recognized and therefore not reported (false-negative)
2. Reporting bias
   - sometimes a reaction is erroneously seen as an adverse reaction and reported (false-positive)
   - publication in the literature leads to more reports on this specific adverse reaction
3. The reporting systems do not give data on the incidence of adverse reactions to drugs because of under-reporting and reporting bias, and because of the absence of reliable data on drug use.

Some physicians report adverse reactions to drugs regularly, but most report irregularly or not at all. In a study on anaphylaxis only 8% of the cases of

drug-induced anaphylaxis were reported to the Netherlands Centre for Monitoring of Adverse Reactions to Drugs [6]. 25 % of physicians reported that they had notified an adverse reaction on at least one occasion during their working period [7]. Over a period of 3 years, 8% had reported an adverse reaction [8]. Only a small number of physicians was unaware of the existence of the reporting system. Despite these studies, it is unknown which percentage of adverse reactions is reported. This percentage is different for each drug and for each adverse reaction. Therefore, it is impossible to generate incidence data or relative risks with reporting data.

1.3.1.2 Exposure databases

1. The PHARMO RLS drug database

The PHARMO RLS drug database was set up by the Department of Pharmacoepidemiology and Pharmacotherapy of the University of Utrecht. It is a drug database of pharmacy dispensing histories obtained from 27 community pharmacies covering all prescription drugs that are dispensed in 6 middle-sized Dutch cities [9]. The drug database started in 1985 with one city, and the other cities joined the system in subsequent years. Since January 1, 1990, all six cities are included, which means that the database contains information on a catchment area of approximately 500,000 people. Due to the high standard of computerization in the Dutch pharmacies, patient records are readily available, and can be anonymously transferred to a central database. Since most patients in The Netherlands fill their prescriptions at a single pharmacy, and since all pharmacies within these cities are included in the database, almost all prescriptions of the people within the catchment area of the database are in the database.

The PHARMO RLS drug database may be linked to the hospital admission data in the six cities as held in a database by the department of Internal Medicine II of the Erasmus University Medical School in Rotterdam. Probabilistic linking is done on the basis of patient characteristics (e.g. date of birth, gender, and general practitioner) [10].

2. GIP

The Dutch Health Insurance Fund Council owns the "Geneesmiddelen Informatie Project", a database with data on the numbers of drugs dispensed by pharmacies in The Netherlands to about one third of all people in the national health insurance system (± 3.2. million). These data are billing data, and contain the insurance number of the patient, gender and date of birth, name of

the drug, dose, and number of units dispensed, and the date of dispensing. The advantage is, that all drugs that are paid for by the insurance are captured in this system. The disadvantage of the system is, that it only covers the lower socio-economic classes, since only people below a certain income level are allowed to participate in the insurance. Also, only drugs dispensed by community pharmacies are recorded and OTC preparations are not included. The database cannot be used for record linkage purposes.

3. IMS

Intercontinental Medical Statistics (IMS) is a commercial company, which owns several databases. It obtains sales figures on drugs from pharmaceutical firms, wholesale distribution, samples of prescribers (general practitioners and specialists), and pharmacies, using electronic medical files and sales data. The disadvantage of sales data from pharmaceutical firms is that these data do not consist of dispensing data. The prescribing data can be quite inaccurate because the samples of prescribers are small. There is also a panel of ±150 pharmacies which deliver their data to IMS. The data from pharmacies are anonymized, but it is possible to see which drugs were dispensed to one patient at one moment in time. The sample of pharmacies will be expanded to 300 pharmacies. The indication for use of the drugs in the database is, however, unknown. To cope with this problem, IMS supports the "Integrated Primary Care Information" (IPCI) database. The data from IPCI are promising, in that these contain prescription as well as morbidity data, and the indication for use. A more extensive description of the IPCI system is given below.

1.3.1.3 Morbidity databases

Apart from the databases on morbidity mentioned below, the Central Bureau of Statistics holds a registry of causes of mortality. Unfortunately, this database is of only limited use for pharmacoepidemiologic studies, due to strict privacy regulations.

1. The Dutch Centre for Health Care Information

The Dutch Centre for Health Care Information has a national registry of hospital diagnoses. In this registry, every admission to every general and university hospital in The Netherlands is recorded. Diagnoses are coded according to the ICD-9-CM. On every admission data on gender, date of birth, admission and discharge are recorded, as well as department admitted to, at least one principal diagnosis (mandatory) and up to nine additional diagnoses

(optional). The principal diagnosis is usually the disease which has led to admission. The additional diagnoses usually consist of events which took place during admission (e.g. thrombosis after an operation). The percentage of additional diagnoses which is reported is unknown and depends on the hospital and the physician. The data are not used for billing purposes, and therefore a bias towards relatively well-paid diagnoses is not to be expected.

2. PALGA

PALGA (Pathologisch-Anatomisch Landelijk Geautomatiseerd Archief or Pathological National Automated Archive) is the national registry for pathological diagnostic research performed in hospitals or laboratories all over the country. It includes all data on all biopsies and specimens taken and investigated by pathologists in The Netherlands, and can give information on the number of diagnoses of certain diseases that are made by studying cell or tissue samples (such as carcinomas), and can link these back to the hospitals the data came from. The advantage of the system is that it covers the whole country, and that all data are collected in the registry, the disadvantage is that it only contains data on diseases which have been confirmed by pathological investigation. The system is potentially useful and has been used for epidemiologic research, but so far not for pharmacoepidemiologic studies.

1.3.1.4. A database with both data on drug exposure and morbidity

IPCI

Integrated Primary Care Information (IPCI) is a database consisting of data from Computerized Patient Records of general practitioners. In order to collect the data for the database, general practitioners making use of the Elias database system are included, and a Postmarketing surveillance module is added to their system. The data on each visit of every patient that are recorded are anonymously transferred to IPCI. The data are extensive and include complaints of the patient, investigations performed and their results, diagnoses made and prescriptions of drugs with their indication. Data are transferred anonymously to a "gate-keeper", only identifiable by the general practitioner via a randomized number. The "gate-keeper" anonymizes the data with regards to general practitioner, and these data enter the IPCI database. The database is controlled by a Supervisory Board, which decides on which studies are allowed to be performed and which are not. Additionally, all general practitioners involved in the system have the right to block data on their patients for an intended study. At the moment, about 75 general practitioners, with

approximately 200,000 patients in their practices provide data for IPCI. Part of these patients rarely visit the general practitioner, which leaves 170,000 to 180,000 patients with regular contacts in the database. This system is especially suited for performing pharmacoepidemiologic studies, since morbidity as well as drug data and indications for prescriptions are provided by the database.

1.3.2. Postmarketing methods

1.3.2.1 Descriptive studies

1. Case reports

A case report is a description of a particular adverse event attributed to a particular drug. Such a description may be important as a signal, and may provide important clinical details, which are useful for the early recognition of an adverse reaction. Unless there has been a positive rechallenge or a special test (skin test, in vitro test) to prove the association between use of the drug and the adverse event, it may be difficult to judge the causal relationship.

2. Case series

In a case series, cases of an adverse event attributed to a certain drug are collected and described. The causal relationship in a case series, as in a case report, is assessed on the basis of individual patient data, and depends on the specificity of the clinicopathological pattern, the temporal relationship between use of the drug and the start of the event, recovery of a reversible event after cessation of the drug and, sometimes, rechallenge with recurrence of the event. Also, the use of concomitant drugs and their temporal relationship to the event is taken into account. Since several cases can be compared in such a way, it is sometimes possible to recognise a particular predisposing factor for a certain adverse effect of a drug. In the absence of a control group, case reports and case series give no insight into the incidence or relative risk of adverse reactions. Moreover, the causal relationship may be difficult to assess.

1.3.2.2. Epidemiologic study designs

In epidemiologic study designs, causality is not judged on an individual patient level, but depends on the association between events in patients and the drugs that are being used within a plausible time window prior to the event. Here, as Hill suggested, the strength of the association, the consistency with other

investigations, the specificity of the effect, a compatible time sequence, the presence of a dose-response relationship, the biologic plausibility of the hypothesis, the coherence with what is known of the natural history and biology of the disease, experimental evidence, and analogy may be of importance [11]. There is discussion about these criteria: a certain exposure may have more than one effect (e.g. smoking), and experimental evidence and analogies may not always be present, but some, e.g. a compatible time sequence, are a sine qua non [12].

There are several study designs in pharmacoepidemiology. A short description of these methods is given below.

1. Cohort study

In a cohort study a group of people is defined according to a certain characteristic, and exposure and several variables of interest are measured. Then the cohort is followed over time until the end of the study period. In the analysis, the proportion of diseased people in the group of exposed people may be compared with the proportion in a group of non-exposed people. The proportions are expressed as a cumulative incidence, and the comparison with the unexposed group is expressed as a relative risk. The frequency of disease may also be expressed, however, as an incidence density during exposure, and compared to the incidence density during non-exposure as the incidence density ratio.

Strengths of this study design are [13]:

1. It allows direct measurement of incidence of disease (i.e. adverse reactions) in the exposed and nonexposed groups.
2. It is less liable to selection bias than a case-control study.
3. The study design is suitable for rare exposure (e.g. new drugs).
4. It is suitable for studying several adverse reactions concurrently.
5. If the study is prospective, data on exposure are unbiased, because they are assessed before the outcome occurs.
6. It can elucidate the temporal relationship between exposure and disease.

Disadvantages of this study design are:

1. It can be expensive and time-consuming (because a large group of people is needed if the adverse effect is rare, and because exposure has to be assessed in the whole study population and not only in a subgroup of this population and because usually a long period of follow-up is required).
2. It is sensitive to selection bias (drop-outs, loss to follow-up and selection of patients with more severe disease who will use a newer and

"safer" drug for a certain reason, e.g. because their disease is very serious, or because they are prone to certain adverse reactions).
3. The cohorts are usually too small for the assessment of rare adverse reactions.
4. The validity of the results can be seriously affected by losses to follow-up.

2. Case-control study

In a case-control study, cases are patients with the adverse event. Controls are people without the event and may be matched to the cases according to age, sex or other variables. With each case, one or several (usually 2 to 4) controls are selected. Subsequently, the use of drugs prior to the adverse event is assessed. A comparison is made between the group of cases and the group of controls with respect to exposure, and expressed as the exposure odds ratio.
A nice example of a successful case-control study was the study which was performed by Herbst et al. on the relationship between the use of diethylstilbestrol by the mother and development of clear cell carcinoma of the vagina in the daughter [14]. In this study only 8 patients and 32 controls were sufficient to show the relationship.

Strengths of this study design are [15]:
1. The design is suitable for studying rare adverse reactions.
2. It is relatively inexpensive compared with other study designs. Exposure does not have to be assessed in a whole cohort, but only in a limited number of cases and controls.
3. It may be performed relatively fast with retrospective data.
4. The design is suitable for studying the relationship with several drugs concurrently.
5. It is particularly well-suited to the evaluation of diseases with long latent periods.

Disadvantages of this study design are:
1. It is sensitive to bias:
   - information bias, e.g. recall bias: in case of severe disease, cases will have a better recall of drug use than healthy controls.
   - selection bias: the selection of cases and controls may be biased and related to their exposure status.
   - confounding bias.
2. The design is not suitable for rare exposure: there is a risk that none of the controls will have used the drug of interest.

3. With this design it is not possible to directly compute incidence rates of disease in exposed and nonexposed individuals, unless the study is population based
4. in some situations, the temporal relationship between exposure and disease may be difficult to establish

3. Cross-sectional study

In a cross-sectional study, morbidity and exposure are measured at the same moment in time. The temporal relationship between exposure and outcome, and duration of use are not assessed. Therefore, the cross-sectional design is rarely used in pharmacoepidemiology.

4. Case-crossover study

In a case-crossover study, the patient serves as his or her own control. According to some, the best representation of the source population of cases are the cases themselves. For a case-crossover study, three points are considered pivotal. First, the study must be dealing with an acute adverse event as the result of a transient drug effect. Second, the effect period must be precisely determined. Third, one must be able to obtain reliable data on the usual pattern of drug exposure for each case, over a sufficiently long period of time. The design is useful when studying an acute reaction to a transient drug effect, and when the selection of controls is a problem. Also, it is not liable to confounding by factors that do not change over time. Although selection of controls is not a problem, selection bias of cases may still occur. Periods with and without the disease are compared with regards to preceding exposure. This design is suitable for studying reversible effects. It is also a suitable design for studying life patterns and their relationship to a certain effect (e.g. exertion as a trigger of myocardial infarction [16]).

1.4 The problem of rare, but serious adverse reactions to drugs

The main problem with all these study designs is, that none of them is suitable in case of a rare disease and a rare exposure, which is often the case when there is a serious adverse event to a newly marketed drug that needs to be investigated.

In this thesis, a special study design and its usefulness for postmarketing surveillance is investigated: the nationwide population based case-cohort design.

In this design two postmarketing systems are used: the Dutch Centre for Health Care Information (morbidity data) and the PHARMO RLS drug database (prescription data). The cohort consists of every inhabitant of The Netherlands during the study period. All patients who have had a certain adverse reaction which was reason for admission to a hospital during the study period are the cases. The reference cohort consists of every inhabitant of the catchment area of the PHARMO RLS drug database in the same period.

The idea of case-cohort studies is not new: it was first suggested by Kupper et al. in 1975 [17], and a method for analysis was, among others, described by Prentice in 1986 [18]. The design of a nationwide population based case-cohort study with the use of a dynamic population instead of a fixed cohort, however, and its use in postmarketing surveillance, have never been examined before.

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2

Scope of the thesis

The studies in this thesis were initiated by the Drug Safety Unit of the Inspectorate for Health Care (the former Netherlands Centre for Monitoring of Adverse Reactions to Drugs), when several problems were encountered which could not be solved by using data from the voluntary reporting system for suspected adverse reactions to drugs. Although such systems are useful for generating signals, they do not have the ability to assess the incidences or relative risks of adverse reactions because of variable under-reporting, reporting biases and the absence of reliable data on drug use. When adverse reactions are common, these have mostly been quantified already in clinical trials. Rare events, however, are mostly encountered for the first time after widespread marketing.

When the occurrence of rare drug-associated events prevent these from being discovered before marketing, there are two commonly used epidemiological techniques possible to study their incidence and relative risk in relation to the suspected drug. When a drug is scantily used, i.e. the exposure is rare, a cohort study may be useful. In this design, a group of users ("cohort") of the suspected drug is followed over time, and the nature and frequency of adverse events in this cohort are compared to those in a similar group of non-users. This design is not suitable for rare adverse events, however. When the drug is often used but the disease is rare, it is mostly advised to use a case-control design. In this design, the exposure to the suspected drug in a group of patients with the disease ("cases") is compared to that in a group of patients without the disease ("controls"). Shortly after marketing, however, and sometimes even later, not only the adverse event may be rare, however, but also the suspected drug may be rarely used. In this thesis we examine whether it is possible to use a case-cohort design under such circumstances.

In this thesis, we focus on two events which are both rare and often caused by drugs, i.e. anaphylaxis and agranulocytosis. In chapters 3 and 4, the detailed clinical characteristics are given of cases of anaphylaxis to trimethoprim and agranulocytosis to trazodone. Subsequently, all reports of anaphylaxis and agranulocytosis, as notified to the Drug Safety Unit since 1974, are presented as case-series on anaphylaxis in chapter 5 and on agranulocytosis in chapter 6. In chapter 7, the method is described for performing a case-cohort study with the data on morbidity and exposure as available in the Netherlands. In chapters 8 and 9, the case-cohort studies on anaphylaxis and agranulocytosis are described. In the general discussion, chapter 10, we present the advantages and limitations of the nationwide population based case-cohort design for pharmacoepidemiology.

Part II

Case reports

3

Anaphylactic reactions associated with trimethoprim

A.M.H. Bijl, M.M. van der Klauw, A.C.M. van Vliet, B.H.Ch. Stricker

Submitted

Summary

Background: Anaphylactic reactions to co-trimoxazole are often ascribed to the sulphamethoxazole component of this antibacterial drug.

Objective: To determine whether the trimethoprim component can be the cause of an anaphylactic reaction.

Methods: An analysis was made of reports on anaphylaxis attributed to trimethoprim, as notified to the Drug Safety Unit of the Dutch Inspectorate for Health Care.

Results: In the period between September 1981 and November 1995, thirteen such reports were received. Nine were classified as *probable anaphylaxis*. Of these, the causal relationship between exposure to trimethoprim and anaphylaxis was classified as *definite* in three reports, and as *probable* in the other six. The remaining four reports were classified as *possible anaphylaxis*. In one of these, the causal relationship was classified as *definite*, and in three as *probable*.

Conclusion: Although anaphylaxis due to trimethoprim seems to be rare, it may be more common than previously thought. Apparently, anaphylaxis to co-trimoxazole is not always caused by sulphamethoxazole.

Introduction

Anaphylactic reactions associated with co-trimoxazole (a combination of sulphamethoxazole and trimethoprim) are a common phenomenon. Because sulphonamides are a frequent cause of allergy, reactions to co-trimoxazole are often attributed to the sulphamethoxazole component. Only few data exist on allergy to trimethoprim, a dihydrofolic acid inhibitor. Until recently, trimethoprim was the antibiotic of first choice in general practice in The Netherlands for the treatment of urinary tract infections.

We describe the case-histories of 13 patients with anaphylactic reactions to trimethoprim alone, which were reported to the Drug Safety Unit of the Dutch Inspectorate for Health Care (DSU).

Material and methods

Between September 1981 and November 1995, 13 cases of anaphylactic reactions attributed to trimethoprim were reported to the DSU. In these 13 cases, reporting physicians, patients, or patient's relatives were asked about the symptoms the patients had experienced, the temporal relationship between ingestion of the tablet and the occurrence of symptoms, the duration of

symptoms, previous use of trimethoprim with or without symptoms, known allergies and other diseases and other medication used at the time of the anaphylactic reaction.

Classification of anaphylaxis

In order to classify reports as *anaphylaxis probable*, *anaphylaxis possible* and *anaphylaxis unlikely*, the method shown in Figure 1 was used [1,2]. Symptoms were recorded in the four main tracts involved in allergic reactions: circulatory, respiratory, gastro-intestinal tract, and skin. Circulatory symptoms were: hypotension, tachycardia, collapse. Respiratory symptoms were: dyspnoea, cough, wheezing, stridor, swelling of uvula or pharynx. Gastro-intestinal symptoms were: nausea, vomiting, abdominal cramps, diarrhoea and urge to defaecate. Skin symptoms consisted of rash, pruritus, and/or edema (of skin or oral or conjunctival mucosa). Symptoms were classified as characteristic of anaphylaxis if two or more tracts were involved, except for a combination of only the gastro-intestinal tract and the circulatory tract. In the latter case, or if only one tract was involved, symptoms were classified as compatible with anaphylaxis. The second important feature in distinguishing anaphylactic from other adverse reactions is the temporal relationship between ingestion of the tablet and the occurrence of symptoms. A time interval of one hour or less was considered characteristic of anaphylaxis.

Assessment of causal relationship

The causal relationship between exposure to trimethoprim and anaphylaxis was classified as *probable* if trimethoprim was the only drug used within one hour before developing anaphylaxis, or if other drugs were continued without recurrence of anaphylaxis (Figure 2). If there had also been a similar reaction to inadvertent rechallenge, the causal relationship was classified as *definite*. Also, if the patient had developed similar or milder symptoms to the same drug on a previous occasion, the causal relationship was classified as *definite*. The causal relationship between exposure to trimethoprim and anaphylaxis was classified as *possible* if the patient had been exposed to more than one potential cause within one hour before anaphylaxis [1-3].

Results

Thirteen patients with an anaphylactic reaction to trimethoprim were reported. These consisted of 12 female patients and 1 male patient, aged 22-68 years, treated because of a urinary tract infection (n=12) or prostatitis (n=1).

Figure 1 Model of classification of anaphylaxis.

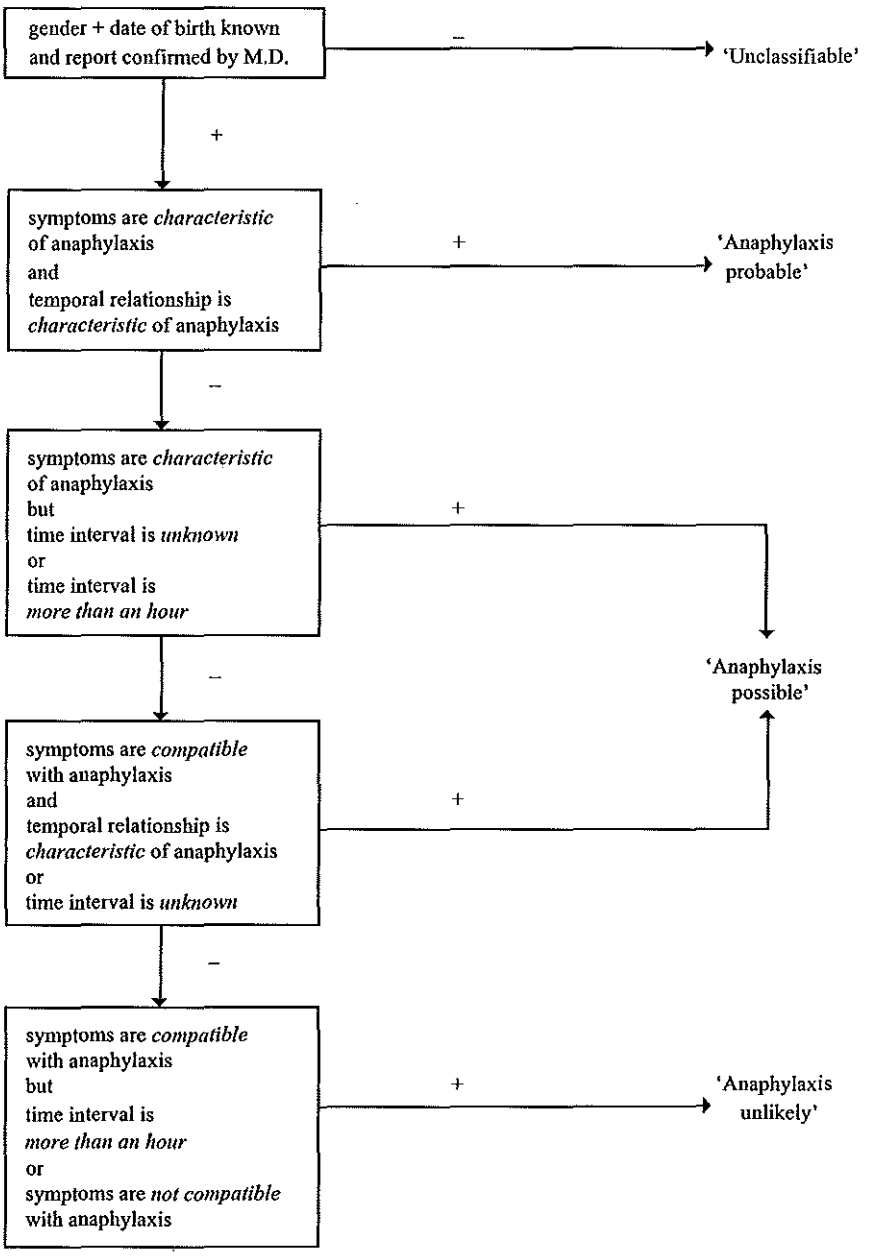

Figure 2. Model of assessment of causal relationship between exposure to trimethoprim and anaphylaxis.

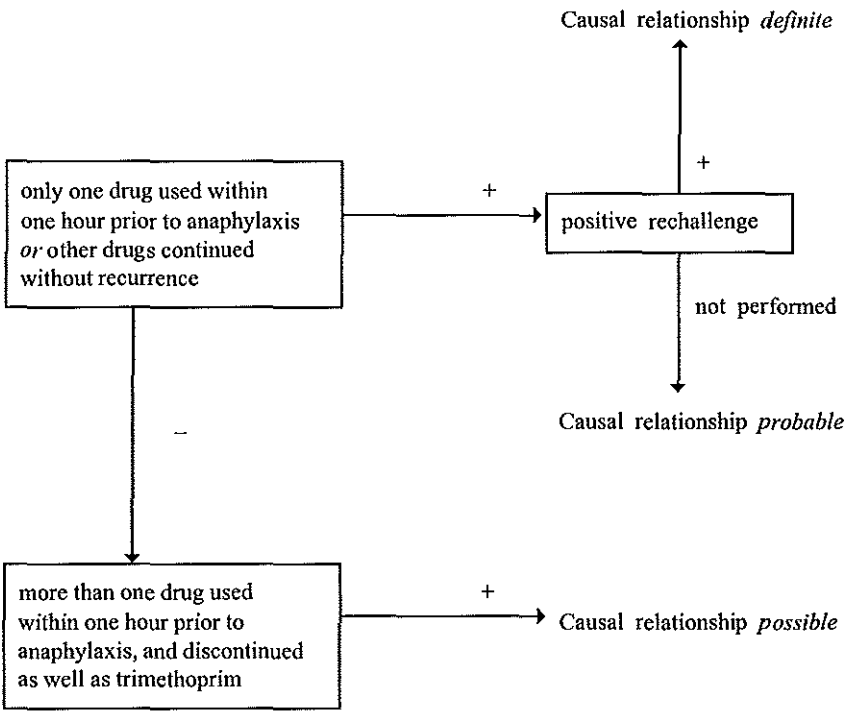

Table 1 shows the relevant characteristics of patients and reactions.

Nine reports were classified as *probable anaphylaxis*. Of these, six patients experienced gastro-intestinal symptoms, eight had skin symptoms, four had respiratory symptoms, and all experienced hypotension. The temporal relationship between ingestion of the tablet and the start of symptoms varied from 5 to 60 minutes. Of these nine patients all but one (M) were known to have used trimethoprim or co-trimoxazole before, three of them reported symptoms such as itch or general discomfort on previous exposure, whereas four had used the drug before without problems.

Patient B had a known allergy to allopurinol, acetylcysteine and latex, patient C to ferrous fumarate, patient F to nitrofurantoin and patient K to penicillin. Patient B was known to have Chronic Obstructive Pulmonary Disease (COPD). Other medication used by these nine patients were: oral

Table 1. Characteristics of reports on trimethoprim-associated anaphylaxis.

Patient	M/F*	Age (years)	Temporal relationship	Tract with symptoms**	Anaphylaxis	Causal relationship	Rechallenge***
A	F	22	15 min.	circ., G-I, skin	*Probable*	*Definite*	+
B	F	67	10 min.	circ., G-I, skin, resp.	*Probable*	*Probable*	+
C	F	30	45 min.	circ., G-I, skin	*Probable*	*Probable*	-
D	F	33	5 min.	circ., G-I, skin, resp.	*Probable*	*Probable*	-
E	F	46	unknown	G-I, skin, resp.	*Possible*	*Probable*	+
F	F	68	30 min.	circ., skin	*Probable*	*Probable*	-
G	F	41	<5 hrs.	skin, resp.	*Possible*	*Probable*	0
H	M	62	unknown	skin, resp.	*Possible*	*Probable*	0
J	F	34	30 min.	circ., G-I, skin	*Probable*	*Definite*	+
K	F	32	60 min.	circ., resp.	*Probable*	*Probable*	-
L	F	29	3 hrs.	skin, resp.	*Possible*	*Definite*	+
M	F	37	30 min.	circ., skin, resp.	*Probable*	*Probable*	0
N	F	37	45 min.	circ., G-I, skin	*Probable*	*Definite*	+

* M = male; F = female
** circ. = circulatory tract; G-I = gastro-intestinal tract; resp.= respiratory tract; skin = skin and conjunctivae
*** Rechallenge: + = symptoms after prior or repeated use; - = prior use without symptoms; 0 = no prior or repeated use.

contraceptives (A,C and N), triamterene/hydrochlorothiazide, beclomethasone-aërosol and salbutamol-aërosol (B). These other drugs had been continued in patients B,C and N.

Four patients were classified as *anaphylaxis possible* because they had symptoms characteristic of or compatible with anaphylaxis, but a temporal relationship between exposure and anaphylaxis which was either unknown or longer than one hour.

Patient E reported similar symptoms after previous use of co-trimoxazole. Patient L had a known allergy to grass pollen. Three patients (E,G, and L) did not use other drugs at the moment of anaphylaxis. Patient H had used trimethoprim for several days when he experienced asthmatic symptoms on day 7 and generalized rash on day 8. The temporal relationship with the use of the tablets was unknown. He was known to have COPD. Other medication consisted of dipyridamole, triamterene/hydrochlorothiazide and phenprocoumon, which were all continued. He was treated with beclomethasone-aërosol, symptoms disappeared in 3-4 days. He had experienced an earlier astmatic reaction after use of a beta-blocking agent. He had not used trimethoprim before.

The causal relationship was classified as definite in patients A, J, L and N because they had experienced allergic symptoms on more than one occasion during use of trimethoprim or co-trimoxazole. Furthermore, other medication was continued without problems in all four patients.

Patient A had taken the first tablet of trimethoprim, and 15 minutes later she became dizzy, was shivering, had blue fingers and red eyes, and was dyspnoeic. She collapsed in the office of the general practitioner, who found a low blood pressure and angioedema. She was treated with clemastine, adrenaline and dexamethasone, and she recovered. Previously, she had felt unwell during use of co-trimoxazole.

Patient N developed generalised urticaria, started vomiting and had diarrhoea, and became hypotensive (systolic blood pressure 70 mm Hg) within 45 minutes after use of trimethoprim. She had used trimethoprim before and developed pruritus at that time, but did not have a reaction as severe as the present one. She recovered quickly after treatment with clemastine and prednisolone.

Patient J developed Quincke's edema, pruritus, erythema, fecal urge, and subsequently collapsed within 30 minutes after using trimethoprim. She had used the drug before without problems. She recovered after treatment with clemastine and hydrocortisone. In patient J, IgE antibodies against trimethoprim were demonstrated. In the other patients, no such tests were performed.

Discussion

Thirteen patients with an anaphylactic reaction attributed to trimethoprim were reported to the DSU. All patients but one were female, which is probably explained by the fact that trimethoprim is often used against urinary tract infections, which have a much higher incidence in women than in men.
Of the thirteen patients, five had known allergies to other substances. We did not find evidence in the literature for a cross-allergy between these substances and trimethoprim.

Ten patients reported use of trimethoprim or co-trimoxazole before, five of them with symptoms. Unfortunately, most patients did not remember which symptoms they had developed, or information was not obtainable.

Little is known in the medical literature of anaphylactic reactions to trimethoprim alone. Although hypersensitivity reactions have been reported, i.e. toxic epidermal necrolysis [4], pneumonitis [5], fixed drug eruption [6-8], vasculitis [9], and photosensitivity reactions [10], anaphylactic reactions were only rarely reported.

There is only one research group that, in examining reactions to co-trimoxazole, tried to separate IgE to sulphamethoxazole and IgE to trimethoprim. They described methods to detect specific anti-trimethoprim-IgE in two patients with an anaphylactic reaction to co-trimoxazole and a positive skin prick test or intradermal test with trimethoprim [11]. The authors suggested that there seemed to be more than one antigenic determinant on the trimethoprim-molecule. Furthermore, they described eight patients with an anaphylactic reaction to co-trimoxazole of whom five had specific IgE to sulphamethoxazole [12]. One had anti-sulphamethoxazole-IgE with a negative RAST-inhibition test and anti-trimethoprim-IgE with a positive RAST-inhibition test. They also found that there are different epitopes on the trimethoprim-molecule, to which specific IgE can be directed [13].

Alonso et al. described three patients, two with anaphylactic reactions and one with generalized urticaria after use of co-trimoxazole, who had positive skin prick tests and rechallenge-tests to trimethoprim and negative skin prick and intradermal tests and rechallenge tests to sulphamethoxazole. No significant levels of specific IgE to trimethoprim could be detected [14].

Cabañas et al. reported on a patient with an anaphylactic reaction to co-trimoxazole, who had a positive skin prick test and specific IgE with a positive RAST-inhibition to trimethoprim, while skin prick and intradermal tests to sulphamethoxazole were negative (as well as in controls) and no IgE against sulphamethoxazole could be found. Oral challenge with sulphamethoxazole was negative, but with trimethoprim this was not performed because of the risk [15].

In one of our reported patients (patient J) anti-trimethoprim-IgE was measured and detected, using two different preparations of trimethoprim.

No data exist on the incidence of anaphylaxis to trimethoprim compared to co-trimoxazole. In a previous study by the Drug Safety Unit [2] 12 anaphylactic reactions were reported to co-trimoxazole (4 *probable* and 8 *possible anaphylaxis*) and 11 to trimethoprim (4 *probable* and 7 *possible anaphylaxis*). Unfortunately however, reporting bias and the absence of reliable drug utilisation figures make it difficult to assess the incidence rates of these adverse effects.

In conclusion, anaphylactic reactions to co-trimoxazole should not be attributed too easily to the sulphamethoxazole-component, since anaphylaxis to trimethoprim may not be as uncommon as previously thought.

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4

Agranulocytosis probably induced by trazodone

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We report on a patient with agranulocytosis probably induced by trazodone, an antidepressant which is chemically different from other antidepressants. With the exception of priapism [1], it has a more favourable adverse reaction profile than conventional antidepressants [2].

Mr. A, a 40-year-old man, was admitted to the hospital because of perianal furuncles, which had developed five days earlier. For one week there was malaise, an unproductive cough, and gingivitis for which he had been treated with 500 mg paracetamol once, sodium perborate mouthwash, and a course of doxycycline. He had no relevant medical history. He had been using trazodone for a month until one week before admission, in a dose of up to 100 mg/day because of depression. He had not been exposed to other drugs or chemicals. On examination he had a normal temperature, mild gingivitis, inguinal lymphadenopathy, and several perianal furuncles. Further physical examination was unremarkable. Hematology profile was normal with the exception of an erythrocyte sedimentation rate of 30 mm/hour (normal range 2-10), leukocyte count of 3.7 x 10^9/l (normal range: 4.0-10.0) and the differential cell count: 74% lymphocytes of which several were atypical, 25% monocytes and 1% eosinophils. Electrolytes and renal and liver functions were normal. Urinalysis showed mild proteinuria and 5-9 leukocytes and 15-19 erythrocytes per high power field. No bacteria were demonstrated, and a urinary culture was sterile. Aspartate aminotransferase was 300 IU/ml (normal: 0-200). Antinuclear factor, Rose-Waaler test, Paul Bunnell and autoantibodies against granulocytes and platelets were negative. A bone marrow biopsy showed a normal erythropoiesis, megakaryopoiesis and myelopoiesis; however, the latter with a slight shift to the left. A culture of the pus of the furuncles yielded a staphylococcus aureus. The patient was isolated for two days and treated with flucloxacillin. No corticosteroids were given. At discharge, four days after admission, leukocytes had risen to 8.4 x 10^9/l (62 % neutrophils) and erythrocyte sedimentation rate had decreased to 19 mm/hour. The urinalysis results were normal, as were results of routine blood examinations performed one week after discharge, and repeated three months later.

In this patient no potential cause of agranulocytosis was found other than trazodone. All other drugs were started after the first symptoms had developed. In view of the temporal relationship, the rapid recovery after discontinuation, and the absence of other causes, trazodone appears to be causative. We could not find any report attributing agranulocytosis or leukopenia to trazodone in the literature, but a decrease in erythrocytes has been attributed to trazodone occasionally [3]. The manufacturer had received two additional reports on agranulocytosis to trazodone.

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Part III

Spontaneous reporting

5

Drug-associated anaphylaxis: 20 years of reporting in The Netherlands (1974-1994) and review of the literature

M.M. van der Klauw, J.H.P. Wilson, B.H.Ch. Stricker

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Summary

Background Since 1963, the Drug Safety Unit of the Dutch Inspectorate for Health Care (DSU) holds a voluntary reporting system.

Objective To analyse all reports received in the years 1974 through 1994, registered as anaphylaxis or as a diagnosis that could contain cases of anaphylaxis.

Methods All reports were classified as probable or possible anaphylaxis according to previously described criteria and the causal relationship between exposure and anaphylaxis was assessed.

Results Nine hundred and ninety-two reports possibly concerning anaphylaxis were received from 1974 through 1994. Fifty-six were unclassifiable. The remaining 936 reports concerned 326 men and 610 women. Three hundred and forty-five reports were classified as anaphylaxis probable, 485 as anaphylaxis possible, and 106 as anaphylaxis unlikely by previously specified criteria. Drugs frequently associated with anaphylaxis (causal relationship certain or probable) were: glafenine (326 reports classified as anaphylaxis probable or possible), combination preparations with (propy)phenazone or propyphenazone/phenacetine (39), diclofenac (30), dextran (20), ibuprofen (14), floctafenine (12), allergen extracts (12), sulphamethoxazole with trimethoprim (12), and trimethoprim (11). There is probably substantial under-reporting as well as reporting bias in these data. Furthermore, many reports were classified as possible and not as probable anaphylaxis because the temporal relationship was unknown or not reported.

Conclusion Drugs that caused anaphylaxis most frequently were glafenine, NSAID and certain antibiotics. Data from a voluntary reporting system such as the DSU are valuable as an early warning system for drugs that may induce anaphylactic reactions.

Introduction

Anaphylaxis is usually defined as an acute allergic reaction that results from the release of pharmacologically active mediators from tissue mast cells and peripheral blood basophils. IgE antibodies bind to mast cells, cause activation and subsequent degranulation. An anaphylactoid reaction is a similar reaction, but not mediated by IgE antibodies, and not requiring previous exposure. Since both syndromes are clinically indistinguishable, in this study we used the term anaphylaxis to describe both [1,2].

Drug-induced anaphylaxis is a very serious adverse event, and may be fatal. Data regarding the incidence of drug-induced anaphylaxis are limited. Antibiotics and radiocontrast agents seem to be the most common causes of serious anaphylaxis, with rates of about 1 in 5000 exposures [2]. Drugs which cause anaphylaxis are mainly known from case reports and some small case series.

Therefore, we present an analysis of all 992 reports of drug-associated anaphylaxis received by the Drug Safety Unit of the Dutch Inspectorate for Health Care (DSU) from 1974 through 1994 and a review of the literature.

Material and methods

Setting

Since 1963, the DSU holds a voluntary reporting system, which has comparable procedures to the Yellow Card System in the United Kingdom. The number of reported reactions per year has grown to 1780 in 1994. Reports are made by physicians (mostly general practitioners). A small number of reports are made by a pharmacist. In this case, the prescribing physician is contacted for more detailed information regarding the adverse event.

In this study, the reports received from 1974 through 1994 were analysed. All reports registered as anaphylaxis, anaphylactic shock or reaction, anaphylactoid shock or reaction, allergic reaction, face oedema, and periorbital or eyelid oedema were included. Also included were combinations of skin reactions (such as rash, urticaria, angioedema or pruritus) with respiratory tract reactions (such as bronchospasm, dyspnoea, laryngeal edema or stridor) or with cardiovascular reactions (such as hypotension, syncope, circulatory failure or collapse) or with death, and combinations of respiratory reactions with death. All reports were classified according to probability of anaphylaxis by the criteria shown in Figure 1 and described earlier [3]. The reports had to contain details on gender and date of birth of the patient, and had to be confirmed by a medical practitioner in order to be classifiable.

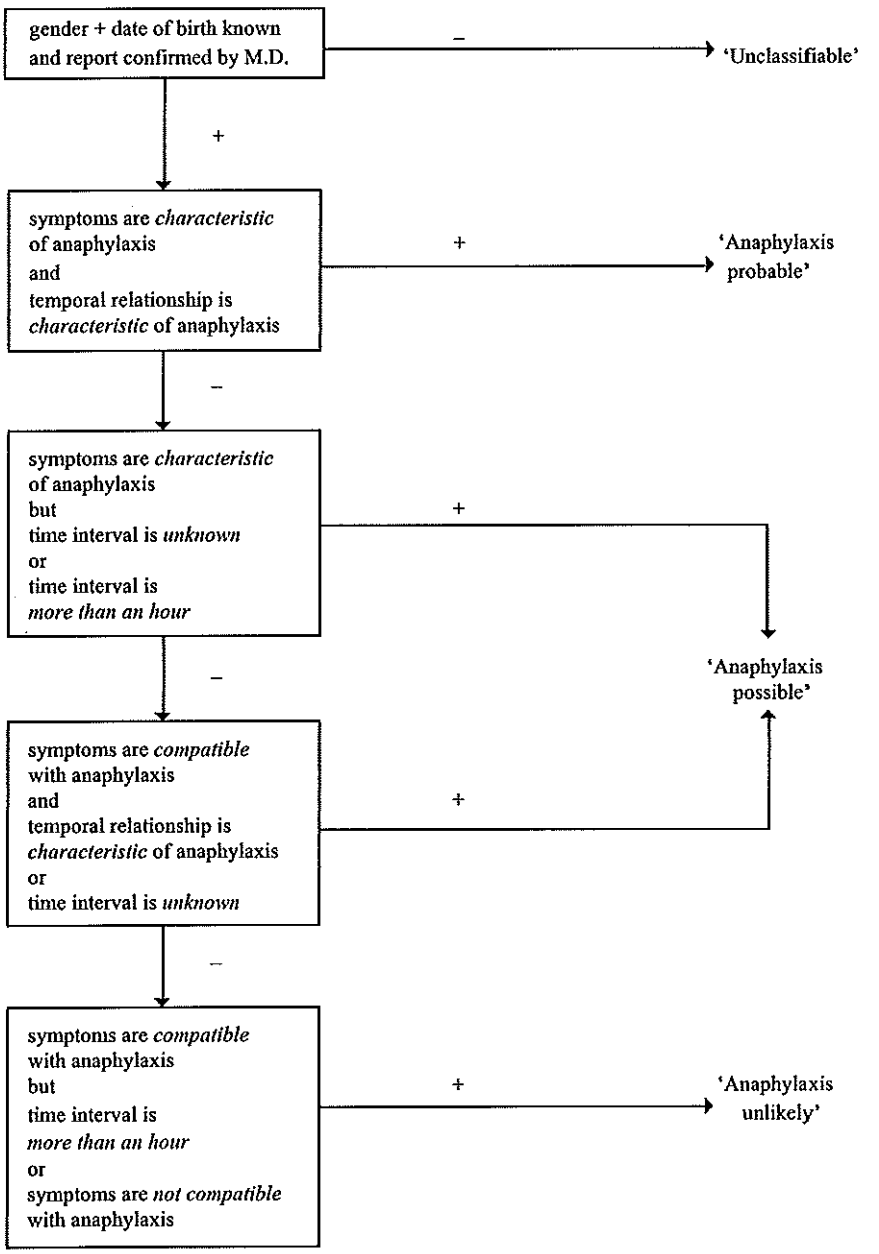
Symptoms characteristic of anaphylaxis were symptoms out of two or more of the following four systems except for a combination of only the cardiovascular and the gastro-intestinal system:

1 Cardiovascular system
2 Respiratory system
3 Skin and conjunctiva
4 Gastro-intestinal system (e.g. nausea, vomiting, diarrhoea, abdominal pain)

Symptoms compatible with -but not characteristic of- anaphylaxis were symptoms out of only one of the above mentioned systems (with the exception of the gastro-intestinal system) or a combination of cardiovascular and gastro-intestinal symptoms. Otherwise, symptoms were not compatible with anaphylaxis [3].

The temporal relationship was considered as characteristic of anaphylaxis if the reaction occurred within one hour after exposure to the causative agent, or was described as "shortly" or "immediately" after exposure.

Figure 1 Model of classification of anaphylaxis.



Assessment of causal relationship

The causal relationship between exposure to the drug and anaphylaxis was classified as "causal relationship probable" if the patient had been exposed to one suspected cause within one hour before developing anaphylaxis, and as "causal relationship certain" if, in addition, there had been a positive reaction to rechallenge. The classification "causal relationship possible" was used if the patient had been exposed to more than one potential cause within one hour before anaphylaxis. The classification "causal relationship unlikely" was used if the temporal relationship was incompatible, i.e. in cases in which the exposure did not precede the reaction (challenge), in which discontinuation was not followed by recovery of a reversible event (dechallenge), or in which continuation or readministration (rechallenge) did not cause a relapse.

Results

Nine hundred and ninety-two reports were analysed, of which 56 were unclassifiable because of lack of data. The remaining 936 reports concerned 345 cases in which anaphylaxis was probable, 485 cases in which anaphylaxis was possible, and 106 cases in which anaphylaxis was unlikely.

Symptoms mentioned in the reports classified as probable and possible anaphylaxis are summarized in Table 1.

Descriptions of reports concerning age and gender, the temporal relationship between exposure and outcome, where and how patients were treated and patient recovery are summarized in Table 2. In those reports classified as probable anaphylaxis, the temporal relationship between exposure and outcome was less than one hour in all reports. Mostly, reports were classified as possible and not as probable anaphylaxis because the exact temporal relationship between exposure and outcome was not reported or unknown, which was the case in 332 out of 485 reports (68.5%).

Of the 830 reports of probable or possible anaphylaxis, 206 patients (24.8%) were admitted to a hospital or treated in the accident and emergency department, 441 (53.1%) were treated by the general practitioner, or not treated at all, and in 117 patients (14.1%) anaphylaxis occurred inside a hospital.

Treatment consisted mostly of adrenaline, corticosteroids, or antihistamines, or a combination of two of these or all three. In 400 reports, treatment was unknown or not mentioned.

Of 345 patients with probable anaphylaxis, two recovered with irreversible damage (one vascular occlusion and one myocardial infarction), and 14 people died due to the anaphylaxis. Of 485 patients with possible anaphylaxis, two still had exanthema at the time of reporting, two recovered with irreversible damage

Table 1. Symptoms mentioned in the reports classified as probable and possible anaphylaxis (% of total in brackets).

Symptom	Anaphylaxis probable (n=345)	Anaphylaxis possible (n=485)	Total (n=830)
Collapse or immeasurable blood pressure	142 (41.2%)	178 (36.7%)	320 (38.6%)
Blood pressure < 100 mm Hg and no collapse, or hypotension during surgery	54 (15.7%)	27 (5.6%)	81 (9.6%)
Blood pressure >100 mm Hg, but lower than normal (hypertensive patient)	6 (1.7%)	6 (1.2%)	12 (1.4%)
Blood pressure lowered, exact value unknown	49 (14.2%)	35 (7.2%)	84 (10.1%)
Blood pressure not lowered	27 (7.8%)	28 (5.8%)	55 (6.6%)
Blood pressure unknown	67 (19.4%)	211 (43.5%)	278 (33.5%)
Urticaria	96 (27.8%)	117 (24.1%)	213 (25.7%)
Erythema or generalised flushing	181 (52.5%)	165 (34.0%)	346 (41.7%)
Angioedema	145 (42.0%)	206 (42.5%)	351 (42.3%)
Pruritus	124 (35.9%)	113 (23.3%)	237 (28.6%)
Other type of skin reaction	10 (2.9%)	18 (3.7%)	28 (3.4%)
Skin reaction of unknown type	1 (0.3%)	4 (0.8%)	5 (0.6%)
No skin reaction	12 (3.5%)	16 (3.3%)	28 (3.4%)
Skin reaction unknown	23 (6.7%)	120 (24.7%)	143 (17.2%)
Edema of the glottis	23 (6.7%)	23 (4.7%)	46 (5.5%)
Bronchospasm	66 (19.1%)	39 (8.0%)	105 (12.7%)
Laryngeal spasm or larygeal edema	13 (3.8%)	3 (0.6%)	16 (1.9%)
Dyspnoea, but type unknown	101 (29.3%)	79 (16.3%)	180 (21.7%)
No dyspnoea	42 (12.2%)	28 (5.8%)	70 (8.4%)
Dyspnoea unknown	106 (30.7%)	316 (65.2%)	422 (50.8%)
Nausea/vomiting	70 (20.3%)	49 (10.1%)	119 (14.3%)
Diarrhoea/fecal urge	45 (13.0%)	24 (4.9%)	69 (8.3%)
Abdominal spasm/stomach pain	33 (9.6%)	20 (4.1%)	53 (6.4%)
No gastro-intestinal symptoms	11 (3.2%)	7 (1.4%)	18 (2.2%)
Gastro-instestinal symptoms unknown	205 (59.4%)	366 (75.5%)	571 (68.8%)

Table 2. Descriptives.

		Anaphylaxis probable (n=345)	Anaphylaxis possible (n=485)
Gender	male/female	124/221	176/309
Age (yr)*	males	47 (35-61)	45 (33-60)
	females	44 (31-60)	48 (34-60)
Temporal relationship	< 15 minutes	149 (43.2%)	40 (8.2%)
	15 - 30 min.	88 (25.5%)	21 (4.3%)
	30 - 60 min.	41 (11.9%)	17 (3.5%)
	"within 1 hour" or "directly after administration"	67 (19.4%)	22 (4.5%)
	1 - 2 hours		29 (6.0%)
	> 2 hours		24 (4.9%)
	not reported or unknown		332 (68.5%)
Admission	admitted to hospital	102 (29.6%)	90 (18.6%)
	treated in A&E**	7 (2.0%)	7 (1.4%)
	treated by GP*** or not treated	158 (45.8%)	283 (58.4%)
	during admission	54 (15.7%)	63 (13.0)
	not reported or unknown	24 (7.0%)	42 (8.7%)
Treatment	adrenaline	5 (1.4%)	4 (0.8%)
	corticosteroid drug	22 (6.4%)	20 (4.1%)
	antihistaminic drug	31 (9.0%)	31 (6.4%)
	adrenaline and corticosteroid drug	15 (4.3%)	11 (2.3%)
	adrenaline and antihistaminic drug	12 (3.5%)	12 (2.5%)
	corticosteroid and antihistaminic drug	45 (13.0%)	39 (8.0%)
	adrenaline, corticosteroid and antihistaminic drug	19 (5.5%)	11 (2.3%)
	other combination	56 (16.2%)	31 (6.4%)
	no treatment	26 (7.5%)	40 (8.2%)
	treatment unknown or not mentioned in report	114 (33.0%)	286 (59.0%)
Recovery	complete	319 (92.5%)	444 (91.5%)
	incomplete or with irreversible damage	2 (0.6%)	4 (0.8%)
	died	14 (4.1%)	7 (1.4%)
	unknown	10 (2.9%)	30 (6.2%)

* median age (25%-75%)
** A&E = Accidents and Emergency department, admission <0.5 day
*** GP = General Practitioner

(one myocardial infarction and one suffered a cardiac arrest and had post-resuscitation rib fractures and amnesia), and 7 died. The drugs responsible for fatal anaphylaxis were: radiocontrast agents (8), dextran (3), glafenine (2), immunotherapy for allergy (2), protamine (1), penicillin (1), tetracosactide (1), metoprolol (1), erythromycin (1) and butylscopolamine with metimazole (1).

One hundred and ninety-nine out of 345 patients with probable anaphylaxis had used the causative drug before, and of them 89 had had a similar reaction to the drug previously, or had a positive rechallenge to the drug. The reactions occurring previously mostly consisted of a less serious reaction (e.g. erythema or urticaria) than anaphylaxis, but sometimes they consisted of a full-blown anaphylactic reaction. Additionally, two patients experienced anaphylaxis during immunotherapy for allergy. Of the 485 patients with possible anaphylaxis, 230 had used the drug before, and of these 94 had had a previous reaction, or had a positive rechallenge. Also, two additional patients experienced anaphylaxis during immunotherapy for allergy. Of the reports of probable anaphylaxis, 26 patients had never used the drug before, and in 120 reports this was unknown. For the reports of possible anaphylaxis these figures were 27 (never used before) and 228 (unknown).

In the reports classified as probable anaphylaxis, the causal relationship between exposure and anaphylaxis was certain in 77 reports, probable in 254 reports, possible in 13, and unlikely in 1 (Table 3). In the reports classified as possible anaphylaxis, the causal relationship between exposure and anaphylaxis was certain in 80 reports, probable in 362 reports, possible in 42, and unlikely in 1.

Table 3. Classification of anaphylaxis and causal relationship.

	Anaphylaxis probable	Anaphylaxis possible	Total
Causal relationship certain	77	80	157
Causal relationship probable	254	362	616
Causal relationship possible	13	42	55
Causal relationship unlikely	1	1	2
Total	345	485	830

The most frequent causes of drug-associated anaphylaxis are specified in Table 4 for the reports classified as probable and possible anaphylaxis.

Table 4. Causes of drug-associated anaphylaxis with a causal relationship certain or probable. (Number of deaths between brackets).

Drug	Anaphylaxis probable (n=331)	Anaphylaxis possible (n=442)	Total (n=773)
Glafenine	146 (2)	180	326 (2)
Propyphenazone/paracetamol, propyphenazone/phenacetine or propyphenazone/carzenide	17	20	37
Diclofenac	12	18	30
Dextran	9 (2)	11 (1)	20 (3)
Ibuprofen	5	9	14
Floctafenine	9	3	12
Allergen extracts	7 (2)	5	12 (2)
Sulphamethoxazole with trimethoprim	4	8	12
Trimethoprim	4	7	11
Amoxycillin	6	3	9
Blood expanders (gelatine)	3	5	8
Naproxen	2	6	8
Mebhydroline	2	4	6
Polidocanol	5	1	6
Cimetidine	2	4	6
Paracetamol +/- coffein	1	5	6
Ranitidine	2	3	5
Tolmetine	3	2	5
Fytomenadione	2	2	4
Chlorhexidine	5	2	7
Furoxone	-	4	4
Nitrofurantoin	-	4	4
Ketoconazole	2	2	4
Atracurium	2	2	4
Amidotrizoic acid*	3 (2)	1	4 (2)
Omeprazole	-	3	3
Metimazole/butylscopolamine	-	3	3
Domperidone	3	-	3
Tetracosactide	2	1 (1)	3 (1)
Indomethacin	1	2	3
Acetylsalicylic acid	1	2	3
Combinations with ergotamine**	1	2	3
Expectorants, combination preparations	1	2	3

Drug	Anaphylaxis probable (n=331)	Anaphylaxis possible (n=442)	Total (n=773)
Acetylcystein	-	3	3
Terfenadine	2	1	3
Metrizoic acid*	2 (2)	1	3 (2)
Patent blue	1	2	3
Metimazole/pitophenone/phenpiveriniumbromide	-	2 (1)	2 (1)
Vitamin B complex	1	1	2
Heparin	2	-	2
Phenazone/phenazonesalicylate/coffein/kinine	-	2	2
Ticlopidine	-	2	2
Parenteral ferri preparation	-	2	2
Urapidil	-	2	2
Captopril	-	2	2
Enalapril	-	2	2
Hydrochorothiazide with potassium sparing drugs	2	-	2
Terbinafine	-	2	2
Methoxsalene	-	2	2
Ergometrine	-	2	2
Medroxyprogesterone	2	-	2
Nalidixinic acid	1	1	2
Pipemidinic acid	2	-	2
Amoxycillin with clavulanic acid	1	1	2
Piroxicam	-	2	2
Ketoprofen	1	1	2
Prilocain	1	1	2
Bromocriptine	-	2	2
Maprotiline	-	2	2
Mefloquine	1	1	2
Bromhexine	2	-	2
Promethazine	2	-	2
Protamine	1 (1)	1	2 (1)
Joxitalamic acid*	2 (1)	-	2 (1)
Diagnostic radiopharmaca	2	-	2
Miscellaneous (all mentioned once)	41 (2)	76 (2)	117 (4)
Total	331	442	773

* = radiocontrast agents
** = ergotamine/cyclizine/coffein or ergotamine/cyclizine or ergotamine/caffein

The causative drugs are shown for those reports in which the causal relationship was classified as certain (n=157) or as probable (n=616). Glafenine was the drug most frequently associated with anaphylaxis, followed by combination preparations of propyphenazone, phenazone and phenacetine, non-steroidal anti-inflammatory drugs (NSAID) (diclofenac, ibuprofen, and naproxen), dextran, floctafenine, allergen extracts, and antibiotics (trimethoprim with or without sulphamethoxazole, amoxycillin, furoxone, and nitrofurantoin).

Discussion

This study describes 992 cases of drug-associated anaphylaxis which were reported during a period of good 20 years. This amounts to a mean of ± 50 reports of drug-associated anaphylaxis per year in a country with 13,000,000 to 15,000,000 people in those years. Probably, the frequency of drug-associated anaphylaxis is much higher, as even though anaphylaxis is a serious adverse reaction, under-reporting is substantial. In two studies performed by the authors only 8% [3] and 4% [4] of cases of drug-associated anaphylaxis had been reported to the DSU. The clinical pattern of anaphylaxis in our study is in line with the medical literature on this topic. Because serious reactions are more readily reported, however, the prevalence of serious symptoms in our study (e.g. shock) may be over-represented. Of 773 reports of drug-associated anaphylaxis with a causal relationship certain or probable, 326 were associated with glafenine, the next most frequently reported drugs being combination preparations of paracetamol and (propy)phenazone and phenacetine (45 reports), diclofenac (30 reports), and dextran (20 reports). The large number of reports on glafenine is especially important in view of the small sales figures the drug had in The Netherlands when compared to the other analgesics and NSAID. Part of this is probably due to reporting bias; part, however, reflects the relatively high incidence of glafenine-associated anaphylaxis, as was found in a nationwide study performed in The Netherlands [3]. Glafenine is an analgesic drug, which was withdrawn worldwide in 1992 [5].

Other drugs often reported as a cause of anaphylaxis were ibuprofen, floctafenine, allergen extracts, the combination of sulphamethoxazole with trimethoprim or trimethoprim alone, amoxycillin, blood volume expanders, naproxen, mebhydroline, polidocanol, cimetidine, ranitidine, and tolmetine. Other antibiotics than the aforementioned were less often reported, partly due to the fact that most reports were made by general practitioners, and probably partly due to under-reporting.

Of 345 reports classified as probable anaphylaxis, symptoms developed later than 30 minutes after exposure in 41 reports (11.9 %). These data support the

policy of observing a patient for at least one hour after administration of antigen during treatment with immunotherapy for allergy.

Out of 830, 21 patients died, and 6 suffered irreversible damage due to the anaphylaxis, stressing the severity of this adverse event.

Unfortunately, because the temporal relationship was not explicitly stated, a relatively large number of reports did not contain enough information for classification as probable anaphylaxis.

Most publications on drug-associated anaphylaxis consist of case-reports. Anaphylaxis associated with almost all of the 17 most frequently reported drugs in Table 4 was described in the literature. Examples are anaphylaxis associated with glafenine [6-11], paracetamol [12-14], diclofenac [15-17], dextran and other blood volume expanders [18-20], ibuprofen [21], naproxen [22], cimetidine [23-24], ranitidine [25] and tolmetine [26-30]. The DSU published case reports or case series on anaphylaxis associated with glafenine [3,4,31], paracetamol [32], floctafenine [33], polidocanol [34], cinoxacin [35], isoflurane [36], chlorhexidine [37], bromhexine [38], and ketoconazole [39].

Three studies were performed on drug-associated anaphylaxis with the use of reporting systems, two focussing on plasma expanders [40,41], and one on NSAID's [42]. In the Swedish study [40] all reports on dextran-induced anaphylaxis during the period 1970-1979 were studied. Since 1974 reporting of fatal and serious adverse reactions has been mandatory in Sweden. The authors estimated the incidence of severe anaphylaxis per unit administered to be 0.013% for dextran 40 and 0.025% for dextran 70. In the German study [41], during the period 1969 to 1976 all reports on transfusion emergencies following the administration of colloid plasma expanders dextran, gelatin and starch were studied. Since in Germany reporting of adverse reactions to drugs was not mandatory, an incidence could not be estimated. The study on NSAID [42] was performed with data from the voluntary Adverse Drug Reaction Reporting System (ADRRS), a system administered by the American Academy of Dermatology. There were four reports of drug-associated anaphylaxis during the period December 1980 through July 1983, all associated with zomepirac.

Only a small number of epidemiological studies on drug-associated anaphylaxis have been performed [43-46], probably because anaphylaxis is a rare adverse drug reaction. All patients admitted with a clinical diagnosis of anaphylaxis, anaphylactic reaction or anaphylactic shock at the Mayo Clinic and affiliated hospitals were studied during a 3.5 year period [43]. Only 23 out of 179 cases of anaphylaxis were attributed to drugs in this study. In a study performed by Rudolph et al. [44], reactions to penicillin treatment in patients visiting venereal disease clinics were studied. The incidence of anaphylaxis among these patients was 0.04% in 1969, with the incidence of moderate to severe anaphylaxis being 0.018% among treated patients. From data of this survey and previous surveys conducted in 1954, 1959 and 1964 combined, the incidence of moderate to severe

anaphylaxis was estimated at 0.025% of treated patients. The authors estimated the fatality rate to be 0.001% of treated patients. In a study performed by Porter and Jick [45] adverse reactions to drugs during hospitalisation at selected medical wards in the U.S.A. and several other countries were studied. Drug-associated anaphylaxis was reported in 0.04% of all patients (12 out of 32,812 patients, 9 following intravenous therapy). In a Danish study [46], during a 13-year period only 20 cases of anaphylaxis were found in the catchment area of one specific hospital, of which 10 were caused by drugs (7 by penicillin and 3 by aspirin). None of these cases were reported to the national health authorities.

Some multi-centre trials have been performed, examining the incidence of anaphylaxis due to plasma expanders [47-50] and penicillins [51], the more frequent causes of anaphylaxis. Ring and Messmer [47] estimated the incidence of anaphylaxis at 0.033% of units of colloid solutions used in general, of which 0.069% for dextran 60/75, 0.007% for dextran 40, and 0.115% for gelatin. In a multicenter prospective study on polygeline [48] only one patient (0.09%) developed systemic anaphylaxis. In a prospective study on dextran 70 induced anaphylaxis [49] 14 females experienced anaphylaxis (0.24%). In a prospective study on plasma substitutes [50] 43 out of 19,593 patients developed an anaphylactoid reaction (0.219%). The incidence of anaphylaxis due to gelatins was 0.345%, due to dextrans 0.273%, due to albumins 0.099%, and due to amidons 0.058%. Unfortunately, the exclusion criteria applied to the cases were not applied to the control group.

One of the most frequently studied drugs causing anaphylaxis is penicillin. In a review article Weiss and Adkinson [52] reported the frequency of allergic reactions to penicillin to vary from 0.7% to 8% of treatment courses in various studies. According to Idsoe et al. [53] the frequency of anaphylaxis due to penicillin varies from 0.01% to 0.035%. In a study performed by the International Rheumatic Fever Study Group [51] 1790 patients from 11 countries were enrolled in a prospective study to determine the incidence of allergic reactions to monthly intramuscular benzathine penicillin. Four patients experienced anaphylaxis, which means a case incidence of 0.2% and an injection frequeny of 0.012%. One of these four patients died (case fatality rate 0.05%).

Radiocontrast materials are also well-known causes of anaphylaxis. Siegle et al. examined a group of patients who previously reacted with anaphylaxis to intravenously administered ionic high-osmolar contrast material. During the study 291 patients received iohexol, of whom 16 (5.5%) experienced anaphylaxis. It appeared that the more serious the prior reaction, the higher the rate was of repeat reactions [54].

In a study by Matloff et al [55] all systemic reactions during one year due to immunotherapy were included. Out of 27,806 injection visits, 143 resulted in systemic reactions (0.51%), of which 17% needed adrenaline therapy.

Our study shows the overview of reports of drug-associated anaphylaxis in The Netherlands in the past 20 years. For some of the drugs this adverse reaction was unknown at the time of reporting. Most of these associations have been published by the DSU. In some (e.g. glafenine), data of the DSU have been used as a signal for a high frequency of adverse reactions. Because of the low frequency of drug-induced anaphylaxis, this adverse reaction is hard to investigate, which the above overview of the literature shows. Prospective studies are very difficult to perform. Also, these are limited to certain classes of drugs, as e.g. drugs which are used relatively frequently in an easily accessible population, as e.g. blood volume expanders which are exclusively used in a hospital setting. Therefore, a reporting system for adverse reactions as the DSU remains essential for signal generation of unknown and unexpected adverse reactions to drugs. For a reporting system to function properly, several items are of importance. First, doctors should report all serious and unexpected adverse reactions. Second, the possibility of obtaining further details from reporters is essential. Third, all unknown or unexpected adverse reactions should be reported in the medical literature.

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6

Drug-associated agranulocytosis:20 years of reporting in The Netherlands (1974-1994) and review of the literature

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Submitted

Summary

In this descriptive study, all 425 reports were included concerning drug-associated agranulocytosis as registered between 1974 and 1994 in the files of the Drug Safety Unit of the Dutch Inspectorate for Health Care. All reports were analysed as to the probability of agranulocytosis or neutropenia according to previously defined criteria. Subsequently, the causal relationship between exposure and outcome was assessed. It concerned 149 men and 271 women. 112 reports were unclassifiable because age, gender or total number of leukocytes at the time of reaction were unknown. In 100 reports agranulocytosis was probable, in 78 possible, in 8 reports neutropenia was probable, in 20 reports neutropenia was possibe, and in 107 agranulocytosis or neutropenia were unlikely. In the 13 reports of probable agranulocytosis or neutropenia with a certain causal relationship, causative drugs were cimetidine, dipyrone, sulphasalazine, methyldopa, spironolactone, propylthiouracil (2), thiamazole, sulphamethoxazole with trimethoprim, gentamicin, a combination preparation containing aminophenazone, benzylpenicillin and indomethacin. The individual drugs most often reported to cause agranulocytosis or neutropenia were: dipyrone (19), mianserine (15), sulphasalazine (13), sulphamethoxazole with trimethoprim (11), the group of penicillins (9), cimetidine (8), the thiouracil derivatives (8), phenylbutazone (8), and penicillamine (8). Agranulocytosis is a serious and fairly frequently reported adverse reaction. The reporting system of the Drug Safety Unit can be used very well for signal generation concerning adverse reactions to drugs.

Introduction

Although agranulocytosis has many causes, the proportion attributable to drugs is substantial. Agranulocytosis was first associated with drugs by Madison and Squier [1]. Knowledge as to which drugs cause agranulocytosis is mainly based on case-reports. As agranulocytosis is such a rare adverse reaction, only few epidemiologic studies have been performed on this subject. However, it is a very serious reaction, with a case-fatality rate of up to 32% [2]. It is therefore very important to know which drugs are capable of inducing agranulocytosis.
The Drug Safety Unit of the Dutch Inspectorate for Health Care (DSU) received 425 reports of drug-associated agranulocytosis, neutropenia or leukopenia in the years 1974 to 1994. In this paper we give a description of these reports and discuss the proposed mechanism of agranulocytosis of each drug.

Material and methods

The DSU runs a voluntary reporting scheme for suspected adverse reactions to drugs. Reports of the years 1974 to 1994 were used for this study. All reports with a diagnosis of agranulocytosis, granulocytopenia, neutropenia, leukopenia, pancytopenia, bone marrow aplasia or depression, and myeloproliferative disorder were analysed according to a two-step procedure.

Firstly, every report was classified by the first author as to probability of agranulocytosis and the probability that agranulocytosis was caused by a drug according to the following criteria:

In order to be classifiable, age and gender of the patient, and the number of leukocytes during the adverse reaction had to be known. Furthermore, if the total number of neutrophils was unknown and the total number of leukocytes was > $1.5x10^9$/l, the report was unclassifiable. ***Agranulocytosis*** or ***neutropenia*** were considered ***probable***, if the nadir of the total number of neutrophils was ≤ $0.5x10^9$/l or > $0.5x10^9$/l but ≤ $1.5x10^9$/l respectively, and (unless the patient died) the patient recovered after discontinuation of the drug. Bone marrow examination, if performed, had to show normal red cell lines and megakaryocytes. The hemoglobin had to be ≥ 6.5 mmol/l and the number of platelets ≥ $100x10^9$/l. If the nadir of the total number of neutrophils was ≤ $0.5x10^9$/l or > $0.5x10^9$/l but ≤ $1.5x10^9$/l, and the patient recovered after discontinuation of the drug, but the result of bone marrow investigation was unknown, as were hemoglobin and the number of platelets, ***agranulocytosis*** respectively ***neutropenia*** were considered ***possible***. If the report mentioned "absolute" or "total" agranulocytosis, this was interpreted as the complete absence of neutrophils. If there was only a bone marrow investigation on autopsy showing no neutrophils and the absolute number of neutrophils in the peripheral circulation was unknown, the report was nevertheless classified as ***agranulocytosis probable*** if the number of megakaryocytes and red cell precursors were normal. If the total number of neutrophils was > $1.5x10^9$/l, or Hb, Ht or platelets were abnormal (see above), or bone marrow investigation was not consistent with agranulocytosis, agranulocytosis was considered unlikely.

Secondly, the causal relationship between agranulocytosis or neutropenia and the drugs used was classified. The causal relationship between drug and agranulocytosis was classified as probable, if the drug had been used during the 10-day period prior to the first symptoms of agranulocytosis (challenge), if the patient had recovered on discontinuation (if he or she survived) (dechallenge), and if there was only one possible cause of agranulocytosis. If use of the drug had been continued and the patient recovered nevertheless, the drug was not considered as causal. Furthermore, the patient should not have used cytostatic drugs, immunosuppressive agents or radiotherapy within six weeks prior to the onset of agranulocytosis. Also, there should not be a systemic disease which could

have given rise to neutropenia, a neoplasm or granulomatous disease affecting the bone marrow, or any other disease presenting as agranulocytosis. If, in addition, there had been a positive reaction to rechallenge or a positive lymphocyte stimulation test, the causal relationship was classified as certain. If there was more than one possible cause of agranulocytosis, the causal relationship of each of these was classified as possible. In any other case, the causal relationship was classified as unlikely.

Results

In the years 1974 to 1994, the DSU received 425 reports of agranulocytosis (n=225), neutropenia (n=58), or one of the other aforementioned diagnoses associated with drug use (n=142). This group of 425 consisted of 149 men and 271 women. Of 5 persons the gender was unknown. Of these 425, 112 were unclassifiable because age or gender of the patient, or the total number of leukocytes at the time of the adverse reaction was unknown. Of the remaining 313 reports, 100 were classified as agranulocytosis probable, 78 as agranulocytosis possible, 8 as neutropenia probable, and 20 as neutropenia possible, whereas in 107 reports agranulocytosis was considered unlikely, mostly because the clinical picture suggested generalised bone marrow depression. In the further analysis, only the 206 reports of agranulocytosis or neutropenia will be considered. These consisted of 72 men and 134 women, with a median age of 56 (25%-75%: 39-69 years) and 64 years (25%-75%: 48-73 years) respectively. In 25 patients, the course of the adverse reaction was fatal (12%) (Table 1). Three patients, however, after recovery from agranulocytosis, died due to concomitant illnesses. Four patients had not yet fully recovered at the time of reporting. Of ten patients, outcome was not known. In 35 reports, the suspected drug had been used before but this was not stated in the majority of reports. Eleven patients had had the same adverse reaction to the suspected drug before, and in these cases the causal relationship was classified as "certain". Almost 90% of all drugs had been used orally.

Most common symptoms were fever (106 patients), throat ache, pharyngitis or tonsillitis (28 patients), sepsis (27 patients), stomatitis or glossitis (21 patients), pneumonia, bronchitis or respiratory insufficiency (18 patients), exanthema or rash (17 patients), and skin infection, erysipelas or furunculosis (12 patients). Symptoms were not stated in 66 reports.

Causes of agranulocytosis and neutropenia are listed in Table 2, divided by classification (probable and possible), and causal relationship between exposure and outcome. There were 11 reports of probable agranulocytosis and 2 of probable neutropenia in which the causal relationship between drug and event was classified as "certain". The causative drugs were cimetidine, dipyrone,

Table 1. General descriptives of the study population (n=206).

		Agranulocytosis probable	Agranulocytosis possible	Neutropenia probable	Neutropenia possible	Total
Course	died	11	12	1	1	25 (12%)
	fully recovered	85	58	6	18	167 (81%)
	unknown	4	8	1	1	14 (7%)
Suspected drug used previously	yes	17	13	2	3	35 (17%)
	no	-	3	-	-	3 (1%)
	unknown	83	62	6	17	168 (82%)
Previously same reaction to suspected drug	yes	6	3	1	1	11 (5%)
	no	4	7	1	1	13 (6%)
	unknown	90	68	6	18	182 (88%)

sulphasalazine, methyldopa, spironolactone, propylthiouracil (n=2), thiamazole, co-trimoxazole, gentamicin, and a combination preparation containing aminophenazone. The drugs causative of neutropenia were benzylpenicillin and indomethacin. The individual drugs most often reported to cause agranulocytosis or neutropenia were: dipyrone (n=21), mianserin (n=15), sulphasalazine (n=13), co-trimoxazole (n=11), the group of penicillins (n=9), cimetidine (n=8), the thiouracil derivatives (n=8), phenylbutazone (n=8), and penicillamine (n=8).

Discussion

This study reviews all reports of agranulocytosis and neutropenia during 20 years of reporting in The Netherlands. Several drugs are already well-known causes of agranulocytosis, such as the thiouracil derivatives, carbimazole and thiamazole, dipyrone, sulphasalazine, and several anti-arrhythmic agents.

When looking at the numbers of reports of agranulocytosis or neutropenia due to a certain drug, a high number could erroneously suggest a high incidence rate. Firstly, the numbers of reports of agranulocytosis and neutropenia are accumulated over the whole period. As not all drugs were on the market for the full 20 years, cases of agranulocytosis to some drugs had to accumulate over much shorter periods. Secondly, sales figures of drugs differ enormously. If drug A is used more often than drug B, and the percentage of patients developing an adverse event is similar to both drugs, one would expect more reports of adverse events due to drug A than to drug B. This is generally speaking true, but, thirdly, reporting bias may occur. When a drug is newly marketed, for instance, a relatively high percentage of adverse events is reported. This may also occur after a newly recognized adverse reaction is published in the medical literature. When a drug is widely known to cause a certain adverse reaction, however, the number of reported events usually declines. Fourthly, when estimating an incidence rate on the basis of reporting data and sales figures, this is invariably underestimated because of under-reporting and because sales figures are always higher than the real amount of drugs used in the population. Therefore, reporting data cannot be used to compare drugs with respect to the incidence rate of agranulocytosis, but can be used for hypothesis generation.

Few case series as large as this study have been published. This is inherent to the rarity of the adverse reaction. Case reports were published on all drugs listed as being frequently reported to the DSU in this article, e.g. mianserin, sulphasalazine, phenylbutazone, penicillamine, and dipyrone [3], the group of penicillins [4], cimetidine [5], naproxen [6], and the thiouracil derivatives [7,8], but also on drugs that were reported as a cause less frequently, such as clozapine [9-11], cephalosporins [12], procainamide [13], dapsone [14,15], paracetamol [16], and ticlopidine [17]. The DSU published case reports and case series on

Table 2. Causes of agranulocytosis/neutropenia (206 patients, more than one cause per patient possible).

Drug	Agranulocytosis/ neutropenia probable		Agranulocytosis/ neutropenia possible		Total (total agranulocytosis)
	A	B	A	B	
Dipyrone	7	5	4	5	21 (17)
Mianserin	8	2	2	3	15 (13)
Sulphasalazine	6	1	4	2	13 (13)
Co-trimoxazole	5	1	1	4	11 (10)
Anti-arrhythmic agents*	4	1	4	1	10 (10)
Penicillins**	4	1	3	1	9 (8)
Thiouracil derivatives***	4		3	1	8 (8)
Phenylbutazone	2	2	2	2	8 (8)
Cimetidine	1	3	4		8 (7)
Penicillamine	1	2	1	4	8 (7)
Diclofenac		3	3	1	7 (5)
Carbamazepine	2	1	4		7 (5)
ACE-inhibitors#	2	1	3		6 (6)
Hydrochlorothiazide with potassium sparing diuretics		3		3	6 (6)
Indomethacin	1	1		4	6 (3)
Cephalosporins##	1	1	1	2	5 (5)
Oxyphenbutazone	1		3	1	5 (5)
Nitrofurantoin	2	1	1	1	5 (4)
Salicylic acid derivatives		1		4	5 (4)
Clozapine	2	1	2		5 (4)
Carbimazole	1		3	1	5 (2)
Sulphonylurea derivatives###		2		2	4 (4)
Methyldopa	1	1		2	4 (4)
Thiamazole	2		2		4 (4)
Nucleosides				4	4 (4)
Aminoglutethimide	2	1		1	4 (4)
Ibuprofen		2	1	1	4 (4)
Pentazocine		1		3	4 (3)

Drug	Agranulocytosis/ neutropenia probable		Agranulocytosis/ neutropenia possible		Total (total agranulocytosis)
	A	B	A	B	
Levamisole	2		2		4 (3)
Promethazine	2	2			4 (3)
Chloramphenicol		2		1	3 (3)
Paracetamol and combination preparations		3			3 (3)
Perazine		1	1	1	3 (3)
Mebhydrolin	2	1			3 (3)
Ranitidine	1			2	3 (2)
Imipramine	1		2		3 (2)
Other drugs (all mentioned twice or less)[$]	14	13	10	12	49 (42)
Total	81	60	66	69	276 (241)

A= causal relationship certain or probable
B= causal relationship possible

* = procainamide(2), ajmaline (1), tocainide (1), aprindine (5) and amiodarone (1)
** = amoxycillin (1), azlocillin (1), benzylpenicillin (3), phenethicillin (1), cloxacillin (1) and penicillin (2)
*** = methylthiouracil (1) and propylthiouracil (7)
\# = captopril (5) and enalapril (1)
\#\# = cephalexin (1), cephazolin (1), cefuroxime (1), cefotaxime (1) and cephradine (1)
\#\#\# = glibenclamide (1) and tolbutamide (3)
$ = phenytoin (2), chlorthalidone (2), sulphamethizole (2), norfloxacin (2), naproxen (2), clomipramine (2), trazodone (2), omeprazole (2), alimemazine (2), pirenzepine, ticlopidine, ibopamine, hydralazine, nifedipine, spironolactone, nalidixic acid, doxycycline, clindamycin, gentamicin, fusidic acid, dapsone, azapropazone, combination preparations with aminophenazone respectively propyphenazone, sulindac, piroxicam, pirprofen, niflumic acid, allopurinol, glafenine, valproate, levodopa with carbidopa, chlorpromazine, haloperidol, zuclopenthixol, zopiclone, cinnarizine, metronidazole, combination prepararations with pyrimethamine, and theophylline.

agranulocytosis attributed to aprindine [18,19], spironolactone [20], ticlopidine [21], propylthiouracil [22], pirenzepine [23], trazodone [24], and omeprazole [25], and on leukopenia attributed to mianserin [26].

Other Adverse Drug Reaction Reporting Systems have also published case series on agranulocytosis. The Swedish Adverse Drug Reporting Committee published two reports on drug-induced blood dyscrasias [2,27]. Dipyrone and antithyroid drugs seemed to cause agranulocytosis most commonly, but also

sulphonamides were a frequent cause of all types of drug-induced blood cell disorders. The mortality rate among the cases of agranulocytosis was 32%, which is higher than the 12% in our study. This high figure, however, may be due to reporting bias, as serious cases are more readily reported. A case-history study of drug-induced blood disorders was performed at the Group Health Cooperative of Puget Sound, consisting of 225,000 members, over the period 1972 through 1981 [28]. They found 7 hospitalized patients with a granulocytopenia attributable to drugs, of which 4 to sulphasalazine. There were 875 patients using sulphasalazine during this period.

Epidemiologic studies have been performed on several drugs. Arneborn and Palmblad studied 133 patients admitted because of neutropenia in the Stockholm region (1,490,000 inhabitants) over the period 1973-75 [29]. Of these, 45 cases were probably drug-induced, giving an annual incidence of 0.001%. When they repeated this study over the years 1976-77 [30], the mortality rate was 27%, higher than in the first study (2%). The most frequent causes of agranulocytosis were sulphonamides, antithyroid drugs, and phenothiazines. An overview over the period 1973-78 related some of the incidence figures to sales figures [31]. The highest frequency was found for thenalidine, followed by thyreostatics, penicillamine and sulphonamides. Only 35% had been reported to the authorities. The mortality rate was 11%.

The largest study performed was the International Agranulocytosis and Aplastic Anemia Study (IAAAS), an international case-control study, in which cases and controls were collected from 1980-86. 362 community-acquired patients were found, which were compared with a control group. Comparing analgesics, positive associations were found for phenylbutazone, oxyphenbutazone, indomethacin and dipyrone, but there was considerable variation by region for dipyrone, which the authors could not fully explain [32]. The authors also found an excess risk for antithyroid drugs [33]. Comparing anti-infective drugs, positive associations were found for co-trimoxazole and macrolides [34]. When analysing the data for cardiovascular drugs, a positive association was found for propranolol, dipyridamole, digoxin, acetyldigoxin, cinepazide, procainamide, and aprindine [35]. The IAAAS has been criticized in several publications [36-41] with regards to methodology. Ibanez et al. continued the data collection that had started because of the IAAAS and reported on antiarrhythmic drugs over the period 1980-1988. A relevant risk was found only for aprindine [42].

The pathogenesis of agranulocytosis is not clear for many drugs. Two types of mechanisms play a role: the immune type, which is mediated by drug-induced auto-antibodies. The antibodies are directed against mature peripheral granulocytes or against their precursors. The other is the toxic type, leading to direct destruction of myeloid cells. There are two categories: the drug is either directly

toxic or it is toxic due to accumulation of inadequately detoxified or excreted metabolic end products [3,43]. Reaction mechanisms affecting the peripheral blood cells probably result in agranulocytosis more rapidly than mechanisms affecting the bone marrow, since in the latter case there will still be mature cells available in the peripheral circulation for a certain period.

Agranulocytosis induced by pyrazolones, such as aminopyrine or dipyrone are examples of the immune mechanism. Amidopyrine-dependent leukagglutinines were demonstrated by Moeschlin and Wagner [44]. It was shown that an antigen-(drug)-antibody complex reacts with receptor sites of the peripheral cells. Crossreactivity between aminopyrine and dipyrone was shown to exist. Three immunological mechanisms have been postulated [45]. Firstly, formation of a complex consisting of the drug and a macromolecule, which leads to induction of antibodies that bind the drug. The antibody-drug complex is adsorbed by granulocytes (e.g. quinidine). Secondly, binding of the drug to the membrane of granulocytes induces antibodies against the drug, which will destroy the granulocyte (e.g. penicillins, cephalosporins [12,46]). Thirdly, the drug triggers the production of autoantibodies, which react with surface antigens on the target cells without the drug itself being involved in the reaction (suggested mechanism for levamisole, described after prolonged use of propylthiouracil, procainamide, and aprindine [3]).

Agranulocytosis induced by phenothiazine derivatives, e.g. chlorpromazine, is an example of a directly toxic mechanism [43,47,48]. This is an idiosyncratic reaction, although there is a relative dose dependency. If the drug is used again after recovery in smaller doses, there is usually no relapse, unless enough is given for a sufficient time. There are no drug-dependent antibodies. The granulopoietic cells themselves are thought to be more susceptible to the effects of the drug in individuals who develop agranulocytosis to such drugs, since these patients seem to have fewer marrow cells in proliferative cycle [49]. Other drugs thought to cause agranulocytosis by a toxic mechanism are clozapine, phenytoin, and beta-lactam antibiotics [3,50,51].

Conclusions

The drugs most often reported as a cause of agranulocytosis or neutropenia in our study were mianserin, sulphasalazine, co-trimoxazole, dipyrone, the group of anti-arrhythmic agents (notably aprindine), the group of penicillins, cimetidine, the thiouracil derivatives, phenylbutazone, and penicillamine. Despite years of experience, remarkably few epidemiological data exist on this topic. Further study should focus on the incidence rate of drug-induced agranulocytosis or neutropenia and compare them with other drugs used for similar indications.

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Part IV

Case-cohort study

7

The population based case-cohort study in pharmacoepidemiology: a nationwide approach

1 Introduction

Pharmacoepidemiological studies on adverse reactions after marketing of drugs face two specific problems. First, when it concerns serious adverse reactions, the incidence will be low, otherwise the drug would not have been approved for marketing, although there may be exceptions to this rule (e.g. antineoplastic agents). Clinical trials are not suitable for detecting rare (but sometimes serious) adverse effects, since usually only 1000-2000 patients are included in these trials. Second, when studying drugs soon after marketing, the prevalence of exposure to the drug will be low.

Voluntary reporting systems are useful for signal generation, but these systems cannot be used for reliable assessment of incidences or relative risks, due to reporting bias and under-reporting of adverse events. Although several countries record sales of drugs, these figures may give an overestimation of the use of the drug, as actual intake is almost invariably lower. Hence, an incidence assessment based on reporting data and sales figures will always be an underestimation of the actual situation, and will suffer from reporting bias.

Cohort studies are suitable for studying drugs with low exposure rates, but are not very suitable for studying low exposure and rare adverse reactions concurrently. A postmarketing cohort would be suitable for studying low exposure, but in a postmarketing cohort, background incidence figures are needed on the outcome of interest, and these are not always available. There still would be a problem if the adverse reaction is rare as well, since a sizeable cohort would be needed to study the relationship. Case-control studies are suitable for studying rare disease but not rare exposure, and are very often subject to selection and information bias (Table 1).

Table 1. Quantitative study designs in pharmacoepidemiology

	Exposure common	Exposure rare
Disease common	clinical trial	cohort study
Disease rare	case-control study	case-cohort study

In 1975, Kupper, McMichael and Spirtas proposed a new study type: a hybrid retrospective [1], later termed case-referent [2] or case-cohort design [3]. This design was mainly used in studies on occupational health and in cancer prevention trials. As we anticipated that the case-cohort design might have advantages in studying rare events to uncommonly used drugs, we studied

whether it would be suitable for pharmacoepidemiological studies on a nationwide scale.

2 Theoretical considerations

In epidemiology, cohort and case-control designs are probably the most frequently used study approaches. In pharmacoepidemiology, cohort studies in the usual sense are infrequently performed. Unlike cohorts based on a common and stable factor, e.g. persons with a special genetic trait or blood group, drug exposure acts as an 'on-off' risk factor. Hence, people who are exposed at one point are not exposed at another point in time. Therefore, it is important to study drug exposure in a biologically plausible risk period (i.e. compatible with the pharmacodynamic and pharmacokinetic properties of the drug) prior to outcome, in cases and non-cases.

When studying rare diseases, such as anaphylaxis and agranulocytosis, a nationwide study may be needed in order to collect enough cases. Usually, the risk factors for such rare diseases are studied with a case-control design. When the drug of interest is rarely used, however, use of the drug may not show up in controls. Of course, this is not a problem if this exposure is also rare or absent in cases, but it is if many cases are exposed. Then, an exposure odds ratio can only be assessed by artificial methods, for instance by adding ½ to all cells in the 2x2-table.

In the case-cohort design, a cohort is formed on the basis of a common factor, and data are collected prospectively on development of the outcome of interest ("cases"). All cases and a randomly selected sample of the cohort (reference cohort) are used in the study. This reference cohort may contain some cases. In a pharmacoepidemiological case-cohort study, exposure to the drug of interest is determined in cases and compared to that in the reference cohort, and expressed as a relative risk (see below). This design is very efficient if the determination of exposure status or other risk factors is expensive or time consuming (e.g. the assessment of blood levels of a drug). Then specimens can be collected on the whole cohort, and the assessment of these can be done afterwards on the cases and the reference cohort only.

In this thesis, the nationwide cohort is defined as all inhabitants in The Netherlands within the study period. This nationwide cohort consists of a dynamic population rather than a traditional cohort. Because the Dutch population is rather stable, and changes little within the period of, e.g. a year, however, this population can be treated as a cohort. Theoretically, all inhabitants are followed over time and all symptomatic cases of anaphylaxis and agranulocytosis are enrolled.

Ideally, the exposure in cases during the risk period would be compared to the exposure in all inhabitants during a randomly chosen comparable period. Unfortunately, such exposure data are virtually non-existent. Even if available, these almost invariably consist of aggregated summary statistics from health councils or health maintenance organizations on a usually selective proportion of the total population, or on aggregated sales figures from drug manufacturers. Especially when studying a rare disease where drugs are a main causative factor, such as anaphylaxis and agranulocytosis, detailed information on all used drugs at an individual level are required.

In this thesis, it was studied whether a case-cohort design would be a feasible and efficient approach when studying these rare and often drug-induced diseases. Because it comprises serious and life-threatening disorders, most symptomatic cases will be admitted to a hospital. Therefore, we enrolled all admitted cases in our study. As a reference cohort, we took a sample of all pharmacy data in the study period.

2.1 Data on morbidity

In this thesis, admitted cases of anaphylaxis and agranulocytosis were the subject of interest. In The Netherlands, the Dutch Centre for Health Care Information maintains a nationwide computerized register of hospital diagnoses, in which admission data are filed on a continuing basis from all general and university hospitals in The Netherlands. For every admission, data include a scrambled identification number (retraceable by the hospital only) gender, age, date of birth, hospital identification number, department of admission, dates of admission and discharge, one principal diagnosis (mandatory) and up to nine additional diagnoses (optional). Diagnoses are coded according to the ICD-9-CM. Only principal diagnoses in the registry are used. The principal diagnosis is usually the disease which has led to admission. The additional diagnoses usually consist of events which took place during admission (e.g. thrombosis after an operation). Additional diagnoses are not mandatory, and the percentage of additional diagnoses that is recorded differs from hospital to hospital, and from physician to physician. These data are therefore of only limited use for epidemiological studies.

For the studies in this thesis, only those patients who were admitted to a hospital during the study period because of the disease of interest, were included. Diagnoses were validated by reviewing the discharge summary and, if necessary, additional information was requested from the physicians involved in the treatment of these patients.

2.2 Data on exposure

In the case-cohort design in this thesis, a reference cohort is used which consists of all people in the catchment area of a representative sample of all community pharmacies from 6 medium-sized cities and their surroundings in different parts of The Netherlands, comprising a population of approximately 500,000 persons (=3% of the Dutch population). The PHARMO RLS drug database of the University of Utrecht contains all drug dispensing data from these cities. Data available are total drug dispensing histories of all inhabitants of this catchment area, including a patient identifier (which can only be traced to an individual patient name and address via the pharmacy in order to guarantee confidentiality), gender and date of birth of the patient, prescriber identifier, drug names, dispensing dates, dispensed units, drug strength, and units per day used [4]. The end dates are estimated by dividing the number of tablets/capsules dispensed by the prescribed daily dose, and adding the calculated number of days exposed to the dispensing date. With these data, numbers of prescriptions, numbers of exposed individuals, and numbers of days exposed per drug are available. Also, data are available on concurrent medication on an individual patient level. The exposure in this reference cohort is used as an estimation of the exposure in the total cohort, being the total population in The Netherlands.

2.3 Relative risk estimation

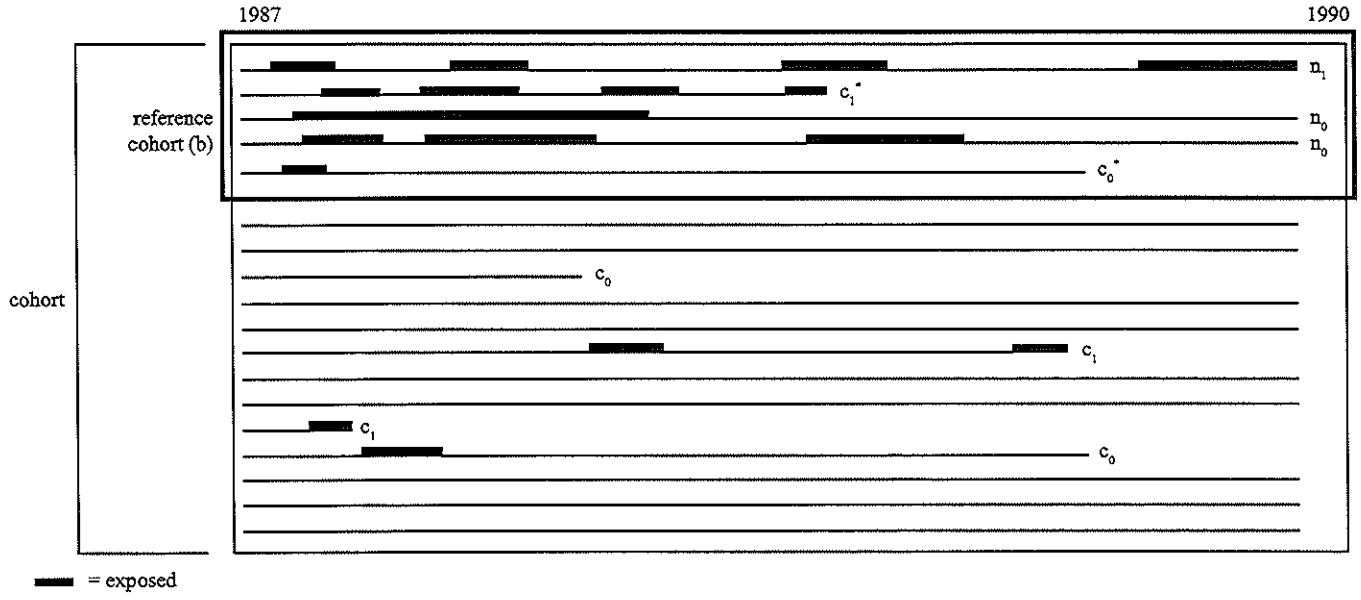
In the current studies, the dynamic population formed by all inhabitants of The Netherlands may be considered as a cohort. Cases were all patients who had been admitted to a hospital with the diagnosis of interest during the study period. All people who were inhabitants of the catchment area of the PHARMO RLS drug database formed the reference cohort (Figure 1).

An estimation was made of the relative risk (RR). This was done by dividing the ratio of cases exposed to a particular drug (c_1) and non-exposed cases (c_0) by the ratio of cohort members exposed to this drug (b_1) and non-exposed cohort members (b_0) as assessed in the PHARMO RLS system [2].

This ratio is given as follows:

$$RR = \frac{c_1/c_0}{b_1/b_0}$$

Figure 1. Case-cohort design.



In the case-cohort design only a sample of the total cohort is used ($b=c^*+n$), but all cases are enrolled (c^*+c). Cases included in the reference cohort are counted twice: once as a case and once in the reference cohort.

b = reference cohort (c^*+n)

c^* = c_1^* (exposed case in reference cohort) + c_0^* (non-exposed case in reference cohort)

n = n_1 (exposed non-case in reference cohort) + n_0 (non-exposed non-case in reference cohort)

c = c_1 (exposed case in total cohort) + c_0 (non-exposed case in total cohort) (this also includes c_1^* and c_0^*)

Miettinen proposed a formula for the standard error of the crude case-cohort risk ratio using a Chi-square statistic [2]. Greenland [5] proposed a formula for stratified analysis using a Mantel-Haenszel formula. Prentice [3] developed a modification of Cox's proportional hazards model for the rate ratio. Nurminen [6] has criticised Miettinen's method in that it would produce too narrow confidence intervals, and Greenland's method in that it would fail if just one of the strata has a zero cell count, and produced an alternative approach using a likelihood procedure. Sato commented on Nurminen's method in that it would fail for sparse stratifications because of the use of the unconditional maximum likelihood [7,8]. He gave a variance estimate of the Mantel-Haenszel risk ratio and a confidence limits method, which is consistent in both large-strata and sparse-data. Schouten et al. proposed a pseudo-likelihood relative risk model, and a similar approach for multivariate rate-ratio estimation [9].

In this study, we used the following formula, which can be used in stratified analysis ([7], see also Table 2):

$R = \Sigma_k b_{0k}c_{1k}/(c_{1k}+c_{0k}+b_k)$
$S = \Sigma_k b_{1k}c_{0k}/(c_{1k}+c_{0k}+b_k)$
$W = \Sigma_k[(c_{0k}+n_{0k})c_{1k}b_{1k}+(c_{1k}+n_{1k})c_{0k}b_{0k}+c_{1k}n_{0k}+c_{0k}n_{1k}]/(c_{1k}+c_{0k}+b_k)^2$

$$RR_{MH} = \frac{R}{S}$$

95%-confidence limits:

$$\frac{2RS+3.84W \pm \sqrt{\{3.84W(4RS+3.84W)\}}}{2S^2}$$

k = 1,2,...,K indexes the strata

Since the number of cases appearing both in the cases (c) as well as in the reference cohort (b) is small compared to the total number of persons in the reference cohort, we did not remove these from the reference cohort in the calculations.

The two studies on anaphylaxis and agranulocytosis were analysed in a different manner. First, in the study on anaphylaxis, a causality assessment was performed on the cases of "anaphylaxis probable" or "anaphylaxis possible",

based on the temporal relationship between exposure and anaphylaxis, the number of drugs used prior to anaphylaxis (one or more than one), and, if performed, on the reaction to a rechallenge with these drugs. Although a very unusual procedure in epidemiology, this strengthened the results of the study, since unlikely causes of anaphylaxis were (rightfully) removed prior to the relative risk assessment. This procedure was not possible in the study on agranulocytosis, since agranulocytosis is not an "on-off" phenomenon, as anaphylaxis is, and the relevant period of exposure prior to agranulocytosis is much longer than the one prior to anaphylaxis.

Second, in the study on anaphylaxis, the risk of developing anaphylaxis to one drug or group of drugs was compared to the risk of developing anaphylaxis to another drug or group of drugs. In the study on agranulocytosis, the exposure among cases was compared to the exposure in the reference cohort. Both methods are valid, and the choice between them depends on the hypothesis of interest. In the study on drug-associated anaphylaxis, due to the short relevant period of exposure prior to anaphylaxis, it was possible to compare drugs in the relative risk estimate, something which was not the case with respect to agranulocytosis. Also, in the study on drug-associated anaphylaxis the aim was to compare risks between drugs (notably glafenine versus other analgesics) something which was not essential in the study on drug-associated agranulocytosis.

Table 2.

	Exposed (E=1)	Non-exposed (E=0)	Total
Reference cohort	b_1	b_0	b
cases	$c_1^*=b_1-n_1$	$c_0^*=b_0-n_0$	$c^*=b-n$
non-cases	n_1	n_0	n
Cases	c_1	c_0	c

In the case-cohort study all cases from the cohort are used. The reference cohort (b) consists of a sample of the total cohort, which may contain some cases (c^*). The cases c^* are the same cases included in the cases (c) and in the reference cohort.

3 Discussion

In a situation of rare exposure *and* rare outcome, the case-cohort design may have advantages. Also, the population based case-cohort design may be

relatively efficient, and less prone to selection and information bias. We will discuss the advantages and limitations of this design in chapter 10.

In our studies, we used pharmacy dispensing data for the assessment of exposure. In contrast to the original case-cohort design, the study base was a dynamic population. Therefore, the cohort is a variable-time or dynamic cohort instead of a fixed cohort because the population in The Netherlands is not constant. We think however, that since the population of The Netherlands did not change substantially during the study period, we may consider this population as a cohort in the classical sense. The exposure, however, is variable over time, since patients will start using drugs and may stop again within the study period. Another important difference between our study design and the traditional case-cohort design is, that our study design is population based and nationwide and that the exposure data are not from a random sample. Even though, however, dispensing data did not come from a random sample of pharmacies, the dispensing data from our sample were representative of drug use in The Netherlands, in that the gender and age distribution of drug users in the sample were similar to that in the Dutch population. It should be emphasised, however, that prescription habits may differ from region to region. In order to minimize this problem, we used exposure data from six cities in different regions in The Netherlands. When aggregated dispensing data from the sample were compared to aggregated sales data from the Dutch Health Insurance Fund Council, they were found to be similar. However, for individual drugs it is still possible that the regional dispensing data are influenced by different prescription behaviour.

Positive misclassification of exposure may occur when patients do not use the dispensed drugs, or do not use all dispensed drugs, but stop taking them when e.g. only half the amount of dispensed drug is finished because their complaints have diminished. Negative misclassification is bound to happen with drugs that are also available over the counter (OTC-drugs), such as several NSAIDs, paracetamol, and antacids. Also, data on drugs dispensed in hospitals or institutions are not available in the PHARMO RLS database. Information bias on exposure in studies with data from this database is not a problem, since data on dispensed drugs are collected before the outcome of interest occurs, and we used data from the pharmacy to validate drug use in cases. Hence, recall bias was excluded.

The outcomes of interest in this thesis were obtained from all general and university hospitals. Positive misclassification of disease could be minimized by validation of the diagnoses. Negative misclassification, occurring if patients were admitted for the disease, but were coded differently, was minimized by including several diagnosis codes in the studies which could have included patients admitted for the disease under study. When patients were not admitted at all, they were missed, and obviously such negative misclassification is

difficult to evaluate. When studying severe adverse reactions, however, the percentage of non-admitted patients will be low, although some may have died without being admitted.

Selection bias might occur, when there would be a selective response by physicians to our requests for admission data (response bias) or when patients with an adverse reaction to one drug were more readily admitted than patients with the same adverse reaction to another drug. The first problem had to be overcome by getting as high a response as possible. The second problem (referral bias) will not be an issue if the design is restricted to severe adverse reactions which always lead to hospital admission, irrespective of previous exposure.

Information bias might occur if data on patients using drug A were better recorded and coded than on patients using drug B. We think that the completeness of data collection depends more on the disease and its severity than on prior drug use, and therefore this should not have jeopardised the validity of the studies.

Adverse events which happened during hospital admission were not included, for two reasons. First, additional diagnoses in the registry of the Dutch Centre for Health Care Information were not used in the study for the reasons mentioned above. Second, the exposure data from the PHARMO RLS database do not cover in-hospital pharmacies and even if available, these data could not be used, because pharmacies inside hospitals in The Netherlands do not have patient-specific, but ward-specific data on exposure.

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8

A population based case-cohort study of drug-associated anaphylaxis

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Summary

1 In order to determine the risk of anaphylaxis as an adverse reaction to drugs, a case-cohort study was performed. Cases consisted of all admissions in 1987 and 1988 to all Dutch hospitals with anaphylaxis as the principal diagnosis, and a random sample of admissions with related symptoms. Hospital discharge summaries were classified according to probability of anaphylaxis by a blinded Audit Committee. Of admissions classified as probable or possible anaphylaxis, the causative agent was assessed. The reference cohort consisted of all persons in the catchment area of a sample of pharmacies in The Netherlands, in the period between January 1, 1987 and December 31, 1988.

2 Out of 934 admissions, discharge summaries on 811 admissions were received, of which 727 contained enough clinical details. Out of 727, 391 were classified as probable or possible anaphylaxis. In 336 of these 391, anaphylaxis was reason for admission. This group consisted of 158 men and 178 women. Drug-associated anaphylaxis occurred in 107 patients.

3 Drug-associated anaphylaxis was most frequently caused by penicillins, analgesics and non-steroidal antiinflammatory drugs (NSAID) with the highest point estimate of the risk relative to all other drugs of 10.7, 6.9 and 3.7 respectively.

4 In the cases of probable anaphylaxis, the risk of anaphylaxis to glafenine relative to all other drugs was 167.7 in 1987 (95%-CI: 63.0-446.4) and 128.6 in 1988 (95%-CI: 50.4-328.5), to amoxycillin 15.2 in 1987 (95%-CI: 5.0-46.0) and 4.4 in 1988 (95%-CI: 1.03-18.9) and to diclofenac 6.1 in 1988 (95%-CI:1.4-26.1).

5 The case-cohort design may be useful for postmarketing surveillance.

Introduction

In many countries information on adverse reactions to drugs is collected by voluntary reporting of adverse reactions, predominantly by medical practitioners and pharmacists. Although voluntary reporting schemes are a useful resource for signal generation, the major limitation is that no insight is obtained into the incidence or relative risk of adverse reactions, due to non-recognition or under-reporting of adverse reactions, false attribution of adverse events to drugs, reporting bias and the absence of reliable drug consumption data. In the past 25 years associations between adverse events and drugs have been reported frequently in the medical literature and/or to reporting systems. The interpretation of such associations and their incidence has posed considerable problems to regulatory authorities. One example concerns the analgesic glafenine, an anthranilic acid derivative which was registered more than 20 years ago in several European,

Asian, South-American and African countries. Since 1972 several cases of anaphylaxis have been reported in the literature [1-16] and several hundreds of cases have been reported to the Netherlands Centre for Monitoring of Adverse Reactions to Drugs. Since a high frequency of reporting does not prove that a particular adverse reaction has a high incidence, other methods than voluntary reporting had to be used to investigate further the hypothesis that anaphylaxis occurs more frequently in response to glafenine than to other analgesics. In a previous study with data of the year 1981 [16], it was demonstrated that the risk of anaphylaxis to glafenine was higher than to any other "prescription only" drug available in The Netherlands. In order to assess whether the situation had changed in recent years after the adjustment of the data sheet in 1986 and in order to compare the risk of anaphylaxis to glafenine with the risk of anaphylaxis to similar drugs, this study was performed.

Methods

Design

In this study a case-cohort design was used [17]. The study base consisted of all persons in The Netherlands in 1987 or 1988 (the base cohort). All dispensing data over the years 1987 and 1988 from the representative sample of pharmacies were considered as a random exposure sample from the base cohort (the reference cohort). This sample was used as the reference source of exposure information. All instances of anaphylaxis in the study base as a reason for admission were defined as cases, of which the drug exposure was compared to that of the reference cohort.

Morbidity data

Anaphylaxis is an acute reaction which occurs within 1 h after exposure to a substance and which can involve the cardiovascular system (e.g. hypotension), the respiratory system (e.g. bronchospasm), the skin (e.g. urticaria) and the gastro-intestinal system (e.g. diarrhoea) [18]. Although immunologically there is a difference between anaphylactic and anaphylactoid reactions, it is clinically not possible to differentiate between both types of reaction, and in the medical literature both mechanisms are referred to as anaphylaxis [19]. Therefore, in this study both types of reaction were included.

Data on morbidity were obtained from a nationwide computerized register of hospital diagnoses, in which admission data are filed on a continuing basis from all general and university hospitals in The Netherlands. Data include gender, date of birth, admission and discharge, one principal diagnosis (mandatory) and up to

9 additional diagnoses (optional) for every admission. Diagnoses are all coded according to the ICD-9-CM. We included all admissions in the study concerning patients who had been discharged in 1987 or 1988 with one of the following principal diagnoses: 995.0 (anaphylactic shock, including allergic shock, anaphylactic reaction and anaphylaxis: 253 admissions), 995.4 (shock due to anaesthesia: 0 admissions), 999.4 (anaphylactic shock due to serum: 3 admissions) and 989.5 (venom; insect stings/bites: 202 admissions). Code 989.5 was included because cases of anaphylaxis to insects could have been classified as an insect sting or bite. Because some cases of anaphylaxis could have been classified under another diagnosis code (e.g. urticaria or allergy), a random sample was obtained of the following principal diagnoses: 693.0 (dermatitis due to drugs and medicaments: 120 admissions), 695.0 (toxic erythema: 36 admissions), 708.0 (allergic urticaria: 60 admissions), 995.1 (angioneurotic oedema: 80 admissions), 995.2 (unspecified adverse effect of drug, medicament and biological: 120 admissions) and 995.3 (allergy, unspecified: 60 admissions).

Exposure data

Data on dispensed drugs were obtained from a representative sample of 28 pharmacies from different parts of The Netherlands with a catchment area comprising a population of approximately 100,000 persons (562,855 prescriptions) in 1987 and 150,000 persons (832,856 prescriptions) in 1988 (=1% of the Dutch population), as kept by the PHARMO exposure database [20]. This population formed the reference cohort. The vital statistics concerning age (stratified in 15-year age groups) and gender in this reference cohort were the same as in the population in The Netherlands (Chi square = 8.1, 11 degrees of freedom; p=0.7). Numbers of prescriptions, exposed individuals and days at risk were used as the denominator in the relative risk assessments. Days at risk were calculated by dividing the total number of dispensed tablets/capsules per patient by the prescribed daily intake of tablets/capsules.

Procedures

The physicians involved in the treatment of the patients with anaphylaxis were asked to provide clinical details at admission by sending an anonymised copy of the discharge summary of the patients in the study. If the copy of the discharge summary did not contain enough details, further information was requested.

An Audit Committee of three experienced consultants in internal medicine assessed the clinical details, blinded with regards to the identity of the patient, physician and hospital, and all possible causes of anaphylaxis. Description of symptoms and signs and of the course of the illness during admission were left unchanged. Every admission was analysed according to a predefined algorithm by

which each admission was classified as either "anaphylaxis probable", "anaphylaxis possible", "anaphylaxis unlikely" or "admission unclassifiable". Discharge summaries had to contain details on gender, date of birth and admission of the patient and symptoms of the reaction. If not available, the admission was judged as "admission unclassifiable". An admission was classified as "anaphylaxis probable" if the patient had suffered from symptoms characteristic of anaphylaxis, i.e. symptoms out of two or more of the following four systems (with the exception of a combination of system 1 and 4) and if the reaction had occurred within one hour after exposure to the causative agent (or had been specified as "shortly" or "immediately" after exposure):

1. Cardiovascular system: collapse, loss of consciousness, hypotension (systolic blood pressure ≤ 100 mm Hg and symptoms characteristic of hypotension or, in case of hypertension, a blood pressure that gave symptoms characteristic of hypotension).
2. Respiratory system: rhinitis, swelling of the uvula or pharynx, laryngeal edema (stridor), bronchospasm (dyspnoea, wheezing, asthma).
3. Skin and conjunctiva: pruritus, erythema, urticaria, angioedema, conjunctivitis.
4. Gastro-intestinal system: nausea, vomiting, diarrhoea, fecal urge, abdominal pain or spasm.

If the patient had suffered from symptoms compatible with -but not characteristic of- anaphylaxis, i.e. symptoms out of only one of the systems 1, 2 or 3 or the combination of system 1 and 4, an admission was classified as "anaphylaxis possible" if the reaction occurred within 1 h or if the time interval between exposure and reaction was unknown. If the symptoms were characteristic of anaphylaxis, but the reaction had occurred later than an hour after exposure or this time period was unknown the admission was also classified as "anaphylaxis possible". In any other case the classification was "anaphylaxis unlikely". Each member of the Audit Committee classified first every individual admission separately. Subsequently, final classification was decided on by consensus or by majority of votes. If no agreement was attained, the admission was classified as "admission unclassifiable". An anaphylactic reaction was classified as severe if it was generalized and potentially life-threatening. This was the case if the patient was suffering from cardiovascular or respiratory symptoms or if there was irreversible damage (e.g. myocardial infarction).

In all cases of "anaphylaxis probable" or "anaphylaxis possible" in which the reaction had been the reason for admission, the causal relationship between exposure and reaction was assessed according to previously defined and accepted criteria [21]. Basically, the exposure-anaphylaxis-association was classified as "causal relationship probable" if the patient had been exposed to *one* suspected cause shortly before developing anaphylaxis, and as "causal relationship certain" if, in addition, there had been a positive reaction to rechallenge. The exposure-

anaphylaxis-association was classified as "causal relationship possible" if the patient had been exposed to more than one potential cause shortly before anaphylaxis. The exposure-anaphylaxis-association was classified as "causal relationship unlikely" if the temporal relationship was incompatible. Also, cases in which the exposure did not precede the reaction (challenge), in which discontinuation was not followed by recovery of a reversible event (dechallenge), or in which continuation or readministration (rechallenge) did not cause a relapse, were classified as "causal relationship unlikely".

Data analysis

An estimation was made of the relative risk (RR) of responding to drug X with anaphylaxis compared to drug Y, classified according to the Anatomical Therapeutic Chemical (ATC) system. Every separate admission classified as "anaphylaxis probable" was considered as a case of anaphylaxis. The relative risk was assessed as follows:

$$RR = \frac{\dfrac{\text{number of cases of anaphylaxis due to drug X}}{\text{number of cases of anaphylaxis due to drug Y}}}{\dfrac{\text{number of prescriptions for drug X in reference cohort}}{\text{number of prescriptions for drug Y in reference cohort}}}$$

Point-estimates were calculated with their 95% confidence intervals [22]. Similarly, relative risk estimations were performed with, in the denominator, the number of exposed individuals and the total number of days at risk per drug. Also, relative risk estimations were performed with, in the denominators of the upper and lower quotient, the number of all cases of anaphylaxis to all drugs except drug X, respectively the total number of prescriptions for all drugs except drug X. In order to see whether this would influence the relative risk estimation, re-analyses were performed with admissions classified as "anaphylaxis possible".

The inter-observer agreement in the Audit Committee was analysed by a weighted kappa test [23].

Results

Physicians responded to requests for information in 811 out of 934 admissions (87%). In 84 admissions the data were insufficient, either due to lack of clinical

details (n=9), or because the consultant refused or failed to co-operate (n=44), or because the patient's file could not be found (n=29) or because the reporting physician did not consider the admission as relevant to this study (n=2).

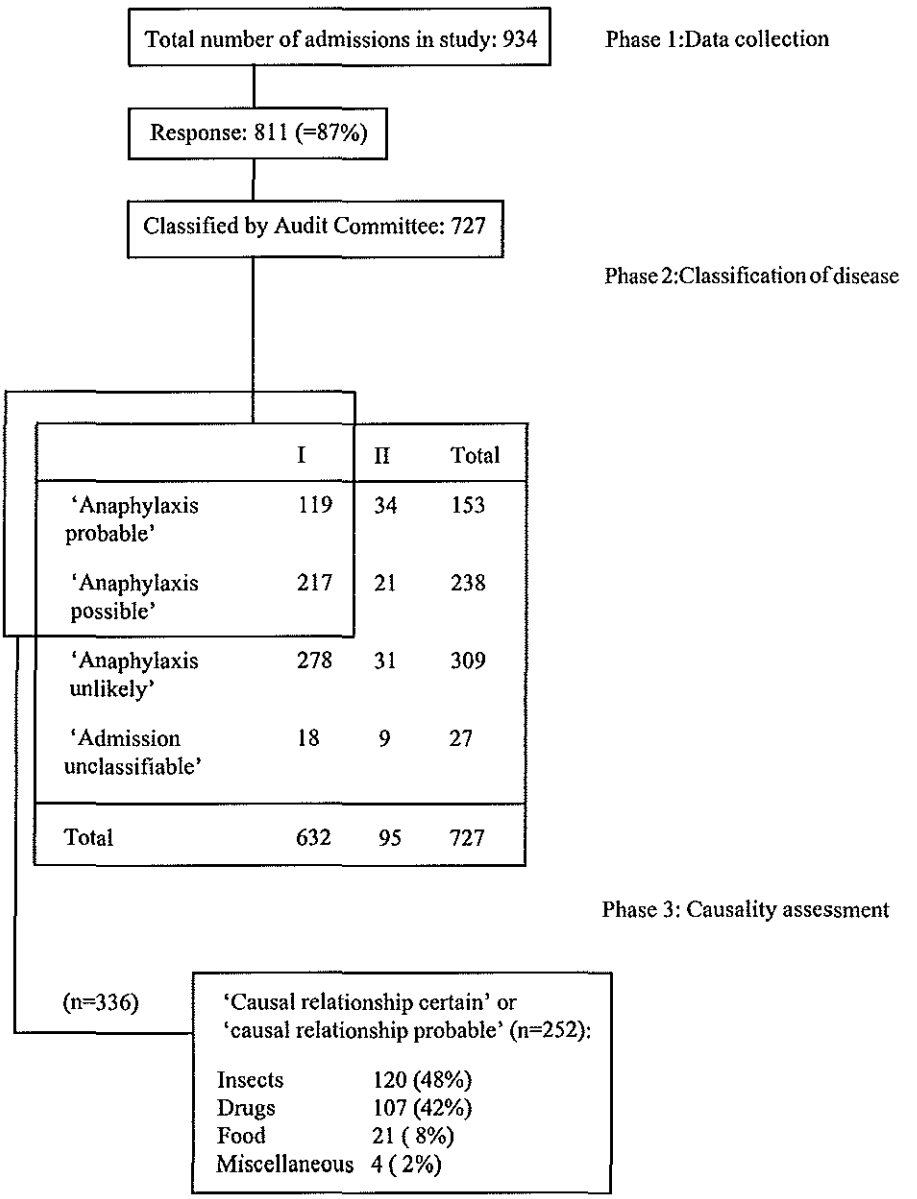
The Audit Committee classified the remaining 727 discharge summaries as "anaphylaxis probable" (n=153), "anaphylaxis possible" (n=238), "anaphylaxis unlikely" (n=309) and as "admission unclassifiable" (n=27). A total of 632 admissions consisted of reactions which occurred outside the hospital and which were the direct reason for admission (community acquired). The remaining 95 consisted either of reactions in the outpatient clinic (n=58) or inside the clinic during admission (n=24) or of reactions on something which was bought or encountered outside The Netherlands and therefore could not be related to drug dispensing data inside The Netherlands (n=3). The place of reaction was not mentioned in the remaining discharge summaries (n=10) and therefore this could not be used to distinguish those cases in which the reaction was the direct reason for admission from the others. Of the 632 reactions which were reason for admission, 119 were classified as "anaphylaxis probable", 217 as "anaphylaxis possible", 278 as "anaphylaxis unlikely" and 18 as "admission unclassifiable" (Figure 1).

Of the admissions coded as diagnoses 995.0 and 999.4, 25 (12%) were classified as "anaphylaxis unlikely" by the Audit Committee. Some of these patients had been admitted with a reaction that was not anaphylaxis but, for example, a Herxheimer reaction, a toxic shock, an intoxication to a drug, collapse after use of nitroglycerin or convulsions. Two were diagnosed as hyperventilation. As a consequence, the definite false-positive misclassification was 12% in these groups. Out of categories 995.0 and 999.4, 174 admissions were classified as "anaphylaxis probable" or "anaphylaxis possible" (85% of the analysed admissions of these diagnosis categories). Of the cases classified as "anaphylaxis probable", 69 came out of the 522 admissions from the other diagnosis categories, mainly from category 989.5 (venom; insect stings/bites). This makes the definite false-negative misclassification 13% in these categories. If category 989.5 was excluded, the false-negative misclassification was only 4% (15 cases out of 356 admissions of which data were received and which were classified by the Audit Committee).

Weighted kappa was reasonably high and remained that way throughout the study (0.55-0.77), which means that the agreement on classification was satisfactory and that the members of the Audit Committee were consistent in their classification.

In the total study there were four deaths: one patient died of a toxic shock, one during a catheter procedure in order to remove stones from the choledochal duct and two patients died of anaphylaxis to intravenously administered contrast media. There were also 21 cases of non-fatal anaphylaxis due to contrast-media.

Figure 1. Overview of study results.



	I	II	Total
'Anaphylaxis probable'	119	34	153
'Anaphylaxis possible'	217	21	238
'Anaphylaxis unlikely'	278	31	309
'Admission unclassifiable'	18	9	27
Total	632	95	727

I = Reason for admission.
II = Occurred in outpatient clinic, clinic, outside The Netherlands or place of reaction unknown.

Only the reactions which occurred outside the hospital and led to admission and which were classified as "anaphylaxis probable" or "anaphylaxis possible" are used in the further analysis. This group consisted of 336 cases, 158 men and 178 women with a median age of 42.5 years (mean 41.0, range 2-84 years, SD 20.4) and 46.5 years (mean 44.9, range 1-90 years, SD 21.5) respectively. Symptoms recorded in the discharge summaries are summarized in Table 1.

Table 1. Symptoms* of 336 patients admitted because of anaphylaxis ("Anaphylaxis probable" (n=119) and "Anaphylaxis possible" (n=217)).

Symptoms	"Anaphylaxis probable"	"Anaphylaxis possible"	Total
Erythema	76 (64%)	115 (53%)	191 (57%)
Angioedema	65 (55%)	107 (49%)	172 (51%)
Hypotension	66 (55%)	56 (26%)	122 (36%)
Bronchospasm	61 (51%)	57 (26%)	118 (35%)
Pruritus	40 (34%)	77 (35%)	117 (35%)
Urticaria	34 (29%)	71 (33%)	105 (31%)
Collapse	42 (35%)	45 (21%)	87 (26%)
Nausea/vomiting	23 (19%)	43 (20%)	66 (20%)
Tachycardia	29 (24%)	34 (16%)	63 (19%)
Loss of consciousness	24 (20%)	22 (10%)	46 (14%)
Diarrhoea	6 (5%)	17 (8%)	23 (7%)
Upper airways**	12 (10%)	9 (4%)	21 (6%)
Conjunctivitis	12 (10%)	9 (4%)	21 (6%)
Laryngeal edema	8 (7%)	11 (5%)	19 (6%)
Abdominal pain	7 (6%)	12 (6%)	19 (6%)
Bradycardia	11 (9%)	4 (2%)	15 (4%)

* There were also 13 patients in the group "anaphylaxis probable" and 9 patients in the group "anaphylaxis possible" with symptoms of cardiac and/or cerebral ischemia, one of them needing mechanical ventilation.

** Rhinitis, oedema of the uvula or pharynx.

In 252 of the 336 admitted cases classified as "anaphylaxis probable" or "anaphylaxis possible" the suspected agent had been classified as "causal

relationship certain" or "causal relationship probable". In those 252, an insect was the cause in 120 cases, a drug in 107 cases, food in 21 cases and in 4 cases it was something else such as a cleaning agent, a burst echinococcus cyst, or exercise-induced anaphylaxis (Table 2).

Table 2. Causes of anaphylaxis leading to admission with a "causal relationship certain" or a "causal relationship probable".

	"Anaphylaxis probable"	"Anaphylaxis possible"	Total
Insects	63	57	120
Food	10	11	21
Drugs			
glafenine	12	8	20
amoxycillin	6	6	12
diclofenac	2	6	8
analgesics with paracetamol (including propyphenazone)	5	2	7
enalapril	-	4	4
co-trimoxazole	-	4	4
dipyrone	1	2	3
nitrofurantoin	1	2	3
carbaspirin calcium	1	2	3
desensitization agents	1	2	3
amoxycillin + clavulanic acid	-	3	3
phenethicillin potassium	1	1	2
nifurtoinol	-	2	2
trimethoprim	-	2	2
naproxen	-	2	2
aspirin	-	2	2
miscellaneous drugs (all mentioned once)	10	17	27
Other causes	1	3	4
Total	114	138	252

Table 3. Relative risks of glafenine, diclofenac, amoxycillins, analgesics, penicillins and NSAID versus all other drugs in 1987.

Drug class (ATC-code in brackets)	Prescriptions	Anaphylaxis hospitalisations			RR (CI 95%) Anaphylaxis probable(A)	Anaphylaxis possible (B)	Anaphylaxis probable or Anaphylaxis possible (C)
	(n = 562,855)	A	B	C			
Glafenine (N02BG03)	1667	6	6	12	167.72 (63.02 - 446.36)	84.01 (34.39 - 205.26)	111.62 (58.18 - 214.15)
Amoxycillins (J01CA04, J01CA54)	10410	4	5	9	15.16 (4.99 - 46.04)	10.63 (4.07 - 27.76)	12.26 (5.94 - 25.30)
Analgesics with paracetamol (N02BE01, N02BE51, N02BE71)	20062	3	1	4	5.41 (1.57 - 18.69)	0.93 (0.13 - 6.86)	2.46 (0.89 - 6.86)
Diclofenac (M01AB05)	7471	0	1	1		2.57 (0.35 - 18.85)	1.58 (0.22 - 11.48)
Penicillins (J01C, J01H, J01J)	14624	4	5	9	10.71 (3.53 - 32.53)	7.51 (2.88 - 19.61)	8.66 (4.20 - 17.88)
NSAID (M01AA, M01AB, M01AE)	21223	2	2	4	3.19 (0.73 - 13.87)	1.83 (0.44 - 7.67)	2.32 (0.84 - 6.47)
Analgesics (N02BA, N02BB, N02BE)	27403	4	3	7	5.58 (1.84 - 16.96)	2.18 (0.66 - 7.17)	3.34 (1.50 - 7.45)

CI 95% = Confidence Interval 95% calculated according to Miettinen [22].

Table 4. Relative risks of glafenine, diclofenac, amoxycillins, analgesics, penicillins and NSAID versus all other drugs in 1988.

Drug class (ATC-code in brackets)	Prescriptions	Anaphylaxis hospitalisations			RR (CI 95%) Anaphylaxis probable(A)	Anaphylaxis possible (B)	Anaphylaxis probable or Anaphylaxis possible (C)
	(n=832,856)	A	B	C			
Glafenine (N02BG03)	2415	6	2	8	128.63 (50.38 - 328.45)	19.63 (4.72 - 81.59)	53.77 (25.54 - 113.17)
Amoxycillins (J01CA04, J01CA54)	18412	2	1	3	4.42 (1.03 - 18.92)	1.23 (0.17 - 8.96)	2.37 (0.74 - 7.57)
Analgesics with paracetamol (N02BE01, N02BE51, N02BE71)	30379	2	1	3	2.64 (0.62 - 11.30)	0.73 (0.10 - 5.35)	1.42 (0.44 - 4.52)
Diclofenac (M01AB05)	13423	2	5	7	6.10 (1.43 - 26.11)	9.54 (3.72 - 24.47)	8.21 (3.73 - 18.08)
Penicillins (J01C, J01H, J01J)	25403	3	8	11	5.02 (1.49 - 16.96)	8.77 (4.01 - 19.17)	7.28 (3.78 - 14.02)
NSAID (M01AA, M01AB, M01AE)	34322	3	6	9	3.67 (1.09 - 12.41)	4.50 (1.88 - 10.79)	4.19 (2.06 - 8.51)
Analgesics (N02BA, N02BB, N02BE)	42683	6	4	10	6.94 (2.72 - 17.74)	2.24 (0.79 - 6.33)	3.78 (1.91 - 7.46)

CI 95% = Confidence Interval 95% calculated according to Miettinen [22].

Of the abovementioned 107 drug-associated cases, glafenine was 20 times the causative agent (19%), amoxycillin 12 times (11%), diclofenac 8 times (7%) and combination preparations with paracetamol 7 times (7%) (of which in 6 cases (6%) propyphenazone was included in the preparation) (Table 2). There were no patients admitted more than once because of drug-associated anaphylaxis during the study-period.

Of the 107 cases 8 (7%) had been reported to the Netherlands Centre for Monitoring of Adverse Reactions to Drugs as cases of suspected drug-associated anaphylaxis, as was determined by comparing birth date, gender and date of admission in both files.

Tables 3 and 4 show the absolute number of anaphylactic reactions per drug or drug group and the risks of anaphylaxis, relative to the total number of prescriptions in 1987 and 1988 separately. Although there is variation per year and per probability class A-C, the point estimates of the relative risks of anaphylaxis to glafenine, amoxycillins, diclofenac, penicillins and analgesics are invariably distinctly greater than one in the group "anaphylaxis probable". The lower relative risks in the groups including cases classified as "anaphylaxis possible" (B and C) may be attributed to non-differential misclassification of disease [24]. The latter resulted from the fact that the group "anaphylaxis possible" included cases in which anaphylaxis could not be excluded but was not very likely either (e.g. ninth-day rash to amoxicillin). Therefore, we concentrated our analysis on the group "anaphylaxis probable". The drug with the highest relative risks was glafenine. In order to assess the risk relative to other well-known causes of anaphylaxis, glafenine was compared with drugs that had been the causative agent of anaphylaxis more than four times in this study. Glafenine was also compared with the group of antiinflammatory and antirheumatic drugs (NSAID), with salicylic acid and its derivatives and, since it is a non-narcotic analgesic, with the total group of non-narcotic analgesics except, of course, for glafenine (Table 5). The relative risk estimation was made on the basis of prescriptions and numbers of patients in the two-year period 1987-1988. Use of glafenine had the highest risk of anaphylaxis in all analyses. When comparing glafenine with combination preparations containing paracetamol the point estimator of the relative risk was (prescriptions and exposed patients respectively) 24.7 - 24.7, with diclofenac 30.7 - 29.6, with NSAID 32.7 - 26.0, with salicylic acid and derivatives 27.7 - 27.0 and with analgesics 20.6 - 18.6. Analysis on the basis of days at risk gave similar figures as analysis on the basis of prescriptions and exposed patients. We also made a comparison with penicillins, since penicillins have a well-known and well evaluated risk of anaphylaxis. This gave rise to a relative risk of 16.8 ($8.5 < RR < 33.3$) (prescriptions) and 20.0 ($10.4 < RR < 38.6$) (exposed patients).

Table 5. Relative risk in the group "anaphylaxis probable" (glafenine versus control drug, 1987 and 1988), estimated on the number of prescriptions and exposed patients.

	Relative Risk (+ 95%-confidence limits)	
Glafenine/Control Drug	Prescriptions	Exposed patients
Glafenine/Analgesics with paracetamol	24.7 (12.8 - 47.8)	24.7 (12.8 - 47.7)
Glafenine/Diclofenac	30.7 (11.8 - 79.9)	29.6 (11.3 - 77.7)
Glafenine/NSAID	32.7 (16.9 - 63.0)	26.0 (13.0 - 52.0)
Glafenine/Salicylic acid and derivatives, incl. combinations	27.7 (10.4 - 73.9)	27.0 (10.1 - 72.3)
Glafenine/Analgesics	20.6 (11.5 - 37.1)	18.6 (10.2 - 33.9)

Discussion

This study shows that drugs and insect stings were by far the most important cause of admission because of anaphylaxis in The Netherlands in 1987 and 1988. Glafenine, amoxycillins and diclofenac were the three most important causes of admission because of drug-associated anaphylaxis. Although there were also 23 cases of contrast-media-induced anaphylaxis, these occurred during admission or in the outpatient clinic, and hence these could not be related to the drug dispensing data from community pharmacies. For this study we considered several types of epidemiological design. Because of the low incidence of anaphylaxis, we did not consider a cohort study as a useful approach. Case-control studies are very suitable for studying rare diseases and are often used when a relationship between the adverse event and a drug is uncertain. In case of anaphylaxis, however, the temporal relationship and clinical pattern usually leave little doubt concerning the causal relationship. We had several reasons for not using a case-control design. Firstly, recall bias would have been introduced into the study. As anaphylaxis is a rapidly developing and impressive type of reaction that patients are not likely to forget, it would not have been easy to find controls subject to the same recall of exposure as cases. Secondly, as drugs are a well-known cause of anaphylaxis, physicians might inquire more insistently about drug use in the index than in the control group. Thirdly, although anaphylaxis is considered to be a rare disease, the low population exposure prevalence of some drugs (e.g. glafenine) could consequently have meant that none of the controls would have been exposed to those drugs. Therefore, we used a case-cohort design, in which the cases were all patients admitted because of anaphylaxis in The Netherlands in 1987 and 1988 and the control cohort was a reference cohort of the Dutch population.

Theoretically, our analysis could have been adversely influenced by three issues. Firstly, diagnostic suspicion bias or referral bias could have played a role. Because of the characteristic pattern and temporal relationship however, and irrespective of the causative agent, anaphylaxis is mostly recognized. It is highly unlikely that general practitioners would recognize anaphylaxis to one drug (e.g. diclofenac) more easily than that to another (e.g. glafenine). Similarly, general practitioners decide on admission of their patients to the hospital on the basis of severity instead of cause. Hence it is unlikely that they would tend to admit more patients with anaphylaxis to one particular type of exposure. This brings us to the second issue: in this study cases of anaphylaxis that were not admitted to a hospital were not included. This could mean that, if anaphylaxis to one drug was more severe than anaphylaxis to another, patients with anaphylaxis to such a drug would have been admitted relatively more often than patients with anaphylaxis to other drugs. However, there are no reasons to believe that anaphylaxis to orally administered amoxycillin, diclofenac or glafenine has a worse prognosis than anaphylaxis to other orally administered drugs. Hence, this will mean that the proportion of community acquired cases of anaphylaxis which leads to admission is more or less the same for these drugs. Thirdly, the reference cohort was not a random sample from the total study base. Random samples are, however, not a goal in itself but used to ensure that the exposure data are representative of the total study base. It is logistically impossible to take a random sample from the total study base of all inhabitants in The Netherlands. Instead of this we used a representative sample of community pharmacies, located in different parts of the country. Therefore, differences in prescribing by medical practitioners in different parts of the country played no role. Thus, we are not aware of any reasons to suggest that the exposure in the reference cohort differs substantially from the exposure in the total study base, especially not as the vital statistics concerning age and gender of the reference cohort were the same as that of the population in The Netherlands.

This study was restricted to principal diagnoses and thus no additional diagnoses have been analysed. The reason is that reporting of additional diagnoses is voluntary. This means that the reporting of additional diagnoses varies per hospital, and it is unknown what percentage of the additional diagnoses is reported. For this reason, in this study the cases of anaphylaxis that occurred inside the hospital were not included. Moreover, the dispensing data from hospital pharmacies are rarely registered on an individual patient level.

The most valid relative risk estimations were made with the group "anaphylaxis probable". This is obvious, in view of the fact that the group "anaphylaxis possible" comprised many non-anaphylactic hypersensitivity reactions. Hence, this introduced a misclassification bias towards a relative risk of one in the combined group of "anaphylaxis possible" and "anaphylaxis probable" [24] (Tables 3 and 4).

Of the group "anaphylaxis probable" 117 out of 119 cases were considered severe on the grounds of involvement of the cardiovascular and/or the respiratory system. For the same reason, of the group "anaphylaxis possible" 125 out of 217 were considered severe. Re-analysis with severe cases did not substantially alter the relative risk comparisons between the drugs. Moreover, since every case in this group has led to a hospital admission, every case should be considered as severe.

The current study confirms the results of an earlier one [16] of a different design. In the current study, besides all cases classified as anaphylaxis, a random sample of cases with other diagnoses was investigated, in order to assess false-negative misclassification. Secondly, all cases were analysed by a blinded Audit Committee in order to prevent classification bias due to knowledge of the cause. Thirdly, relative risk estimations were made with drug dispensing data instead of reimbursement figures from health insurance funds. The former more accurately reflect the consumption of drugs as dispensing data are available on an individual level and as all socio-economic groups are included.

In the current study more cases of anaphylaxis to insect stings were recorded, largely due to the fact that diagnosis code 989.5 (venom; insect stings/bites) was included. The highest risk of community acquired drug-associated anaphylaxis was recorded for the individual agents glafenine, amoxycillin and diclofenac. The risk of anaphylaxis to glafenine relative to penicillins was consistent with the results of the previous study. In the previous study, however, no cases of anaphylaxis to amoxycillin and diclofenac were recorded.

In the absence of reliable outpatient diagnoses this study yields no incidence figures. As the risk of anaphylaxis to penicillins has been estimated at approximately 1:10,000 prescriptions [25-27], and the lower 95% confidence limit of the relative risk versus this group varies from 5 to 10 in the series of analyses, the incidence of anaphylaxis to glafenine may be estimated at 1:1,000 to 1:2,000 prescriptions and hence of diclofenac at approximately 1:10,000 to 1:20,000.

Partly based on the current study, the Committee on Proprietary Medicinal Products (CPMP) of the European Community decided to issue a negative opinion on glafenine, which was supported by 10 of the 12 member states [28]. This advise has been followed by the withdrawal of glafenine in several countries (e.g. The Netherlands, Spain).

We conclude that the case-cohort design -as employed in our study- may be useful for postmarketing surveillance. Unlike a traditional case-control study which requires the enrolment and exposure assessment of controls for every study, the sample cohort of a case-cohort study may be used as a reference for several post-marketing surveillance studies. Moreover, a case-cohort design is suitable for studying rare events to rare exposure. This could make the case-cohort design useful for studying new adverse events in the first two years of marketing of drugs which still have a low exposure prevalence.

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9

A population based case-cohort study of drug-associated agranulocytosis

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Submitted

Summary

In order to determine the risk of drug-associated agranulocytosis as a reason for admission to Dutch hospitals, a population based case-cohort study was performed. Hospital discharge data came from the Dutch Centre for Health Care Information. The reference cohort consisted of all persons in the catchment area of the PHARMO system in The Netherlands, comprising a population of approximately 220,000-484,000 persons in the period 1987-1990.

All admissions in the years 1987-1990 to all general and university hospitals in The Netherlands with agranulocytosis or related diagnoses were included in the study (n=923). A copy was requested of the hospital discharge summaries, and the laboratory and bone marrow results. These were classified according to probability of agranulocytosis without knowledge of exposure status. After ascertainment of diagnoses, the potential causes of agranulocytosis were assessed in all cases which had been classified as probable or possible agranulocytosis.

Discharge summaries were received of 753 admissions, of which 678 contained enough information for analysis. Out of 678, 108 were classified as *agranulocytosis probable* or as *agranulocytosis possible*. In 75 of these 108 cases, agranulocytosis had been the reason for admission. Of these 75 cases, 15 patients had used thiamazole within 10 days prior to developing agranulocytosis, 2 carbimazole, 9 sulphasalazine, 8 co-trimoxazole, 4 clomipramine, and 2 dipyrone with analgesics, yielding adjusted relative risks of agranulocytosis to thyreostatic drugs of 114.8 (95%-CI: 60.5 - 218.6), to sulphasalazine of 74.6 (95%-CI: 36.3 - 167.8), to cotrimoxazole of 25.1 (95%-CI: 11.2 - 55.0), to clomipramine of 20.0 (95%-CI: 6.1 - 57.6), and to dipyrone with analgesics of 26.4 (95%-CI: 4.4 - 111.1).

In conclusion, the highest relative risks were found for thyreostatic agents, co-trimoxazole, sulphasalazine, clomipramine, and dipyrone combined with analgesics.

Introduction

Agranulocytosis is a life-threatening disorder, which frequently occurs as an adverse reaction to drugs [1]. Some drugs are well known causes of agranulocytosis, but there are several drugs of which this is less certain. In the medical literature, case reports continue to appear about agranulocytosis as an adverse reaction to drugs, but the risk of these drugs, expressed as a relative risk or incidence is difficult to estimate. In the years 1980-1986 the International Agranulocytosis and Aplastic Anemia Study (IAAAS) was performed, a population based case-control study involving several study

centres across Europe and in Israel, and encompassing a potential population base of approximately 23 million people [2-18]. We performed a study in The Netherlands for the following reasons: first, in the IAAAS large differences in relative risks between regions in Europe were found, and no epidemiological study has ever included *all* admitted cases of agranulocytosis from a whole country. Moreover, the IAAAS was criticized for potential biases inherent in its design [15,17]. Second, the IAAAS encompassed the years 1980 to 1986. Meanwhile, other drugs have been developed and marketed. We therefore performed a study to assess the relative and attributable risks of drug-associated agranulocytosis in The Netherlands, with a population based case-cohort design.

Material and methods

Setting

Data on morbidity were obtained from the Dutch Centre for Health Care Information, which holds a standardized computerized register of hospital diagnoses. Admission data are filed on a continuous basis from all general and university hospitals in The Netherlands. Whenever a patient is discharged from a hospital, data on gender, date of birth, date of admission and date of discharge, one principal diagnosis (mandatory) and up to 9 additional diagnoses (optional) are anonymously recorded. All diagnoses are coded according to the International Classification of Diseases (ICD-9-CM). At the time of initiation of this study, the most recent years on file available were 1987-1990. In this study, we analysed all records containing potential cases of agranulocytosis, i.e. admissions with the ICD-9-CM codes 288.0 (agranulocytosis), 288.1 (functional disorders of neutrophil polymorphonuclears), 288.2 (genetic anomalies of leukocytes), and 288.9 (unspecified diseases of white blood cells) as principal diagnoses.

Data on dispensed drugs were obtained from the PHARMO Record Linkage System (PHARMO RLS) with an increasing catchment area of approximately 220,000 persons in 1987, 331,000 persons in 1988, 419,000 persons in 1989, and 484,000 persons in 1990. The vital statistics concerning age (overall and stratified) and gender were similar to those of the total Dutch population.

Study design

In this study a population based case-cohort design was used, in which drug use in cases was compared to drug use in a reference cohort [19]. In the case-cohort design, the reference cohort may contain one or more cases. Cases were

patients admitted to a hospital with a validated diagnosis of agranulocytosis. The reference cohort consisted of all people in the catchment area of all pharmacies included in the PHARMO RLS.

Case definition

Agranulocytosis was defined as severe neutropenia ($\leq 0.5*10^9$/l) in an individual 2 years of age or older who used to have normal haematological values, and who had symptoms compatible with agranulocytosis, notably fever and infections. Besides, cases had to comply with all of the following criteria:

- Hb $\geq$ 6,5 mmol/l or Ht $\geq$ 0,32 if normochromic (men and women)
- Platelets $\geq 100*10^9$/l
- Bone marrow aspirate or biopsy which confirmed the diagnosis, or if there was none: recovery of the absolute number of neutrophilic granulocytes within 30 days to $> 1.5*10^9$/l.

Exposure definition

For every case, an index day was defined as the first day of the onset of fever (temperature $\geq$ 38°C), chills or a sore throat. Furthermore, if the symptoms disappeared 5 days before admission or earlier, these were not taken into account. For every case, a risk time window was defined as the 10-day period preceding the index day. It was thus assumed that drug use had to be within 10 days before the index day in order to be a potential hazard. In all cases of a reaction classified as *agranulocytosis probable* or *agranulocytosis possible* the reporting consultant was asked for permission to contact the general practitioner and the pharmacist of the patient in order to assess the use of drugs in the three months prior to admission. These data were used as exposure data, in combination with the data from the patient record. If not available, data on exposure to drugs were collected from the patients' hospital record only. For every drug, the legend duration was calculated by dividing the total number of dispensed tablets/capsules by the prescribed daily number of tablets/capsules. To correct for undercompliance and carry-over effects, this period was multiplied by a factor 1.1 with a maximum of 14 days. Cases were considered exposed to all drugs for which the legend duration fell (partly) within the 10-day risk time window. The drug had to have been used before the onset of agranulocytosis. If the drug was discontinued before the index day, the last day of use of the particular drug had to be within the time period of 10 days before the index day. Since the data from the reference cohort include only data from community pharmacies and not from hospital pharmacies, cases

who developed agranulocytosis during hospital admission (and thus probably due to drugs supplied by a hospital pharmacy) were excluded from the study.

For every member of the reference cohort aged 2 years or older, a random 10-day period was chosen in each year separately. People in the reference cohort were considered exposed to all drugs of which the legend duration fell within this 10-day period. For every drug, the legend duration was calculated as defined above. The average number of users in each year of the study period was calculated in each age and gender stratum, standardised to the population size in the PHARMO RLS catchment area in 1990 (n = 471,812).

Procedures

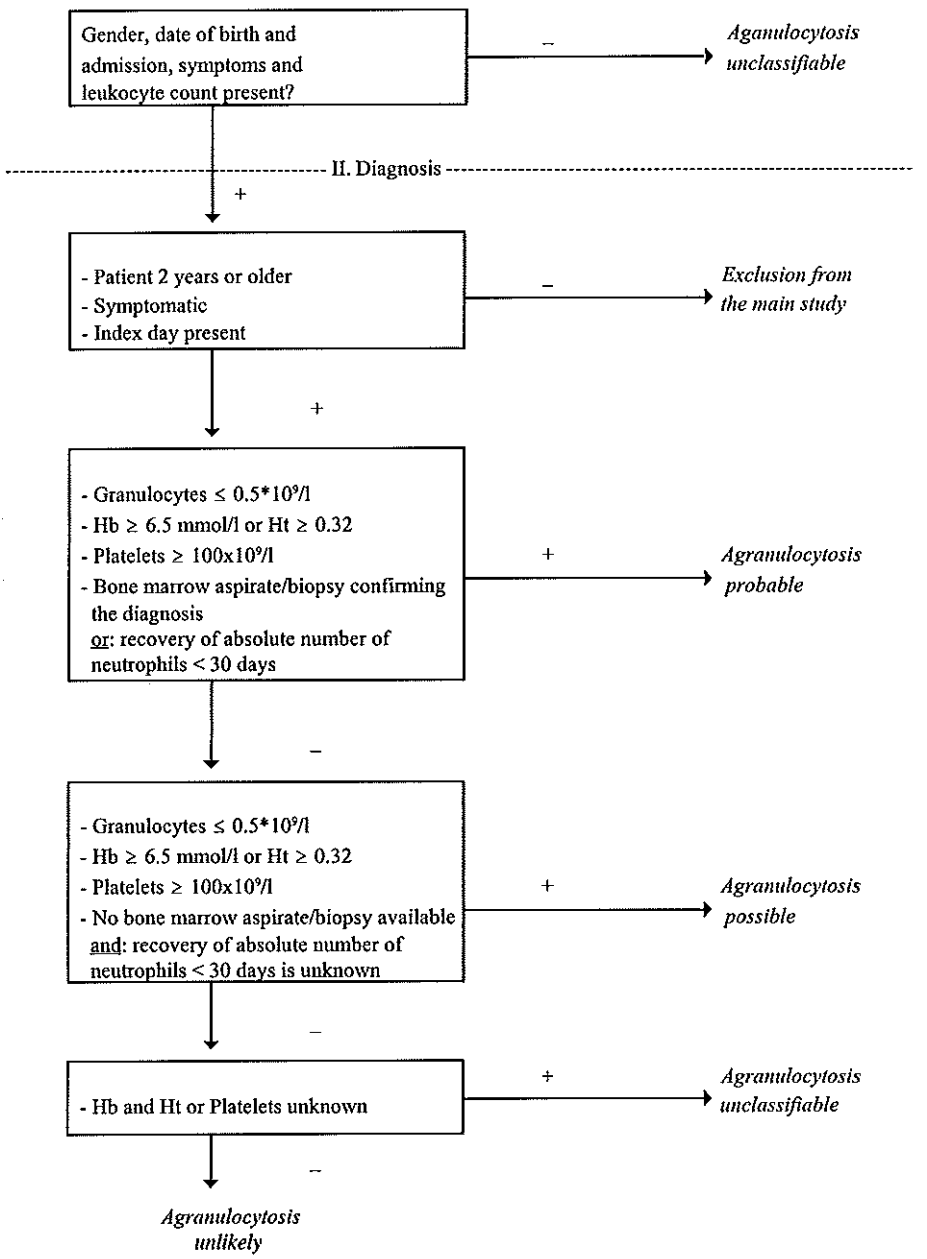
In 1992, a request for information was sent to all hospitals where patients had been discharged in the years 1987-1990 with one of the principal diagnoses mentioned above. All physicians involved in the treatment of these patients received a request for an anonymized copy of the discharge summary, laboratory results and, if available, descriptions of bone marrow material.
If the data received were too scanty further information was requested. All patient data were analysed, blinded with respect to the cause of agranulocytosis, as follows:
Every admission was analysed according to a predefined algorithm, and classified as either *agranulocytosis probable*, *agranulocytosis possible*, *agranulocytosis unlikely* or *agranulocytosis unclassifiable* (Figure 1). A blinded Hematology Review Committee assessed the clinical details of those admissions, where the diagnosis was not straightforward. If two members differed in their opinion on the classification of an admission, it was discussed in a joint meeting of the Committee. Then, final classification was based on consensus (same classification by all three members) or on majority of votes in case of a minor discrepancy (e.g. *agranulocytosis possible* versus *agranulocytosis unlikely*). If no agreement was obtained, the admission was classified as *agranulocytosis unclassifiable*. Furthermore, a 10% random sample of the remainder of admissions was reanalysed by one of the members of the Hematology Review Committee in order to check the validity of the first analysis.

An admission because of agranulocytosis was classified as severe if the patient developed sepsis or septic shock due to agranulocytosis.

Data analysis

An estimation was made of the relative risk (RR) of developing agranulocytosis when being exposed to a certain drug(group) compared to not being exposed. This was done by dividing the ratio of cases exposed (c_1) and

Figure 1. Model of classification by the Hematology Review Committee



not exposed (c_0) to drug(group) X to the ratio of cohort members exposed (b_1) and not exposed (b_0) to this drug(group) as assessed in the PHARMO RLS system [20].

$$RR = \frac{c_1/c_0}{b_1/b_0}$$

Point-estimates were calculated with their 95% confidence intervals for case-cohort studies [20, 21].

The etiological fractions and excess risks were calculated according to standard procedures [22]. All causes which were significantly associated with agranulocytosis in the univariate analysis were subsequently adjusted for age, gender and concomitant drug use in a stratified analysis [21].

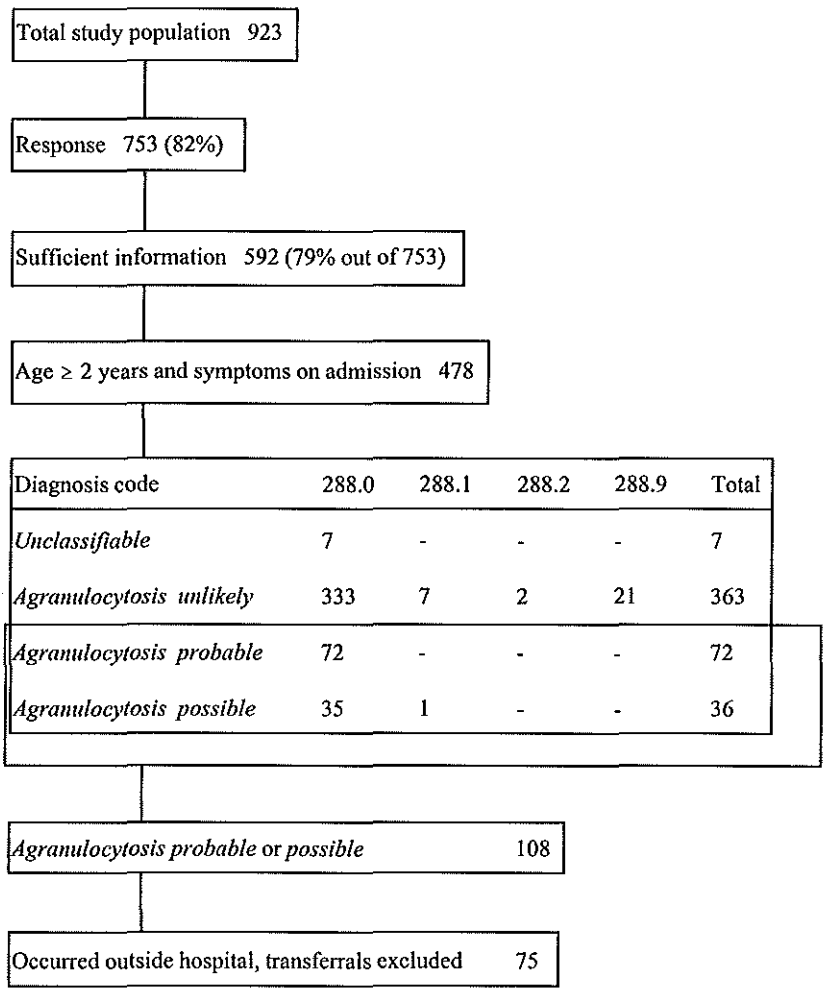
Results

In the period 1987-1990 there had been 923 admissions with a principal diagnosis coded as agranulocytosis (288.0) (n=859), functional disorders of neutrophil polymorphonuclears (288.1) (n=26), genetic anomalies of leukocytes (288.2) (n=2) and unspecified diseases of white blood cells (288.9) (n=36). To the request for information a reaction was received on 753 admissions (82%). On approximately 50% of the cases all relevant information was received (i.e. at least a copy of the discharge summary, and the laboratory and bone marrow results). In the remainder, the hospitals were asked for additional information, resulting in data on 678 admissions, of which 66 concerned patients who had been admitted more than once. A further 86 cases were excluded because of insufficient data (e.g. no description of symptoms or leukocyte counts) and 114 were excluded, either because the patient was less than 2 years old, or had no symptoms on admission (i.e., agranulocytosis was discovered by coincidence).

The remaining 478 admissions were classified as follows: *agranulocytosis probable* (n=72), *agranulocytosis possible* (n=36), *agranulocytosis unlikely* (n=363) and *agranulocytosis unclassifiable* (n=7) (Figure 2). 78 of the 108 admissions classified as *agranulocytosis probable* or *agranulocytosis possible* were reactions which occurred outside the hospital and which were the direct reason for admission. The remaining 30 consisted either of reactions in the outpatient clinic or occurred inside the clinic during admission.

Of the admissions coded as diagnosis 288.0 (agranulocytosis) 333 (74.5% of classified admissions) were classified as *agranulocytosis unlikely* (Figure 2).

Figure 2. Overview of study results.



Most of these patients had been admitted with pancytopenia or a combination of leukocytopenia with anemia or thrombocytopenia due to chemotherapy.

Only the events which occurred outside the hospital and led to admission and which were classified as *agranulocytosis probable* or *agranulocytosis possible* were used in the further analysis, as cases occurring in the hospital could not be related to the exposure data acquired from community pharmacies.

This group consisted of 78 cases. Of this group 6 patients died (7.7%), and in an additional 6 patients the event was severe in view of development of sepsis or septic shock. Fever was present in an additional 66 patients, often with chills.

Blood cultures were performed in 65 patients, of which 39 were positive. Bone marrow was examined in 47 patients, and in 44 confirmed the diagnosis agranulocytosis. In three patients the results were inconclusive. Once the cause of agranulocytosis was discontinued, neutrophil count recovered within 30 days in 43 patients, it did not recover within this period in eight patients, there were no data in 22 patients, and five other patients died before recovery of their neutrophil count.

Five patients were admitted twice, two of these on separate occasions. Three patients, however, were transferred from one hospital to another for the same diagnosis. These three admissions were therefore excluded.

After exclusion 75 cases remained, 30 men (median age 48.5 years, 25% - 75%: 32 - 67 years) and 45 women (median age 61 years, 25% - 75%: 42 - 73 years).

The incidence of agranulocytosis was estimated at 1.7 per million inhabitants in 1987, 2.2 per million in 1988, 2.5 per million in 1989, and 1.6 per million in 1990.

In the cases classified as *agranulocytosis probable* or *agranulocytosis possible*, in which the event had been the reason for admission, the main drugs used prior to the index day were thiamazole (n=15), digoxin (n=12), prednisone (n=10), sulphasalazine (n=9), co-trimoxazole (n=8), paracetamol including combinations (n=8), furosemide (n=6), hydrochlorothiazide with potassium-sparing drugs (n=6), levothyroxine (n=5), ibuprofen (n=5), acenocoumarol (n=5), propranolol (n=5), and oxazepam (n=5). The prevalence of use in the reference cohort (catchment area of PHARMO RLS) is also given in Table 1 for drug groups and in Table 2 for the individual drugs most frequently used prior to agranulocytosis. The relative risks of hospital admissions because of agranulocytosis, adjusted for age, gender, and concomitant drug use, are also shown in Table 1 and Table 2 respectively, as well as the etiological fraction and excess risk for those drugs for which the adjusted relative risk was significantly elevated.

Table 1. Drug-associated agranulocytosis: drugs used prior to index day in 1987-1990 (n=75), drug groups, number of users standardised to the population in 1990. The risk of agranulocytosis expressed as relative risk with 95% confidence interval (adjusted for age, gender and concomitant drug use), etiological fraction, and excess risk per 1,000,000 persons during 10 days exposure.

Drug groups	Number of cases	Number of users in PHARMO RLS	Adjusted relative risk (95%-CI)	Etiological fraction	Excess risk
Thyreostatics	17	752	114.8 (60.5 - 218.6)*	0.23	4.85
Diuretics	17	22095	2.3 (1.2 - 4.3)*	0.13	0.06
Benzodiazepines	15	29824	1.5 (0.8 - 2.9)	-	-
Glucocorticoids	12	5011	8.2 (4.2 - 16.0)*	0.14	0.34
NSAID's	12	20220	2.5 (1.3 - 4.9)*	0.10	0.08
Paracetamol, including combinations	8	14003	2.4 (1.1 - 5.2)*	0.06	0.07
Acetylcysteine/bromhexine/carbocisteine	7	7745	3.9 (1.6 - 8.8)*	0.07	0.15
Coumarins	7	6547	2.7 (1.1 - 6.5)*	0.06	0.09
Penicillins	6	10697	3.1 (1.3 - 7.9)*	0.05	0.11
Salicylates	5	4748	3.6 (1.3 - 9.3)*	0.05	0.14
Calcium antagonists	5	5444	1.9 (0.7 - 5.1)	-	-
Nitrates	5	5499	1.9 (0.6 - 4.9)	-	-

Table 2. Drug-associated agranulocytosis: drugs used prior to index day in 1987-1990 (n=75), individual drugs, number of users standardised to the population in 1990. The risk of agranulocytosis expressed as relative risk with 95% confidence interval (adjusted for age, gender and concomitant drug use), etiological fraction, and excess risk per 1,000,000 persons during 10 days exposure.

Individual drugs	Number of cases	Number of users in PHARMO RLS	Adjusted relative risk (95%-CI)	Etiological fraction	Excess risk
Thiamazole	15	315	230.9 (120.4 - 453.5)*	0.20	10.13
Digoxin	12	5108	5.9 (2.8 - 12.6)*	0.13	0.23
Prednisone	10	1587	19.9 (10.1 - 43.7)*	0.13	0.91
Sulphasalazine	9	523	74.6 (36.3 - 167.8)*	0.12	3.57
Co-trimoxazole	8	1952	25.1 (11.2 - 55.0)*	0.10	1.19
Clomipramine	4	714	20.0 (6.1 - 57.6)*	0.05	0.99
Dipyrone with analgesics	2	325	26.4 (4.4 - 111.1)*	0.03	1.36
Carbimazole	2	376	16.7 (2.6 - 69.7)*	0.03	0.84
Carbamazepine	2	1590	5.9 (1.0 - 24.4)	-	-
Amitriptyline	2	1266	4.0 (0.7 - 16.9)	-	-
Promethazine	2	3024	3.6 (0.6 - 15.1)	-	-

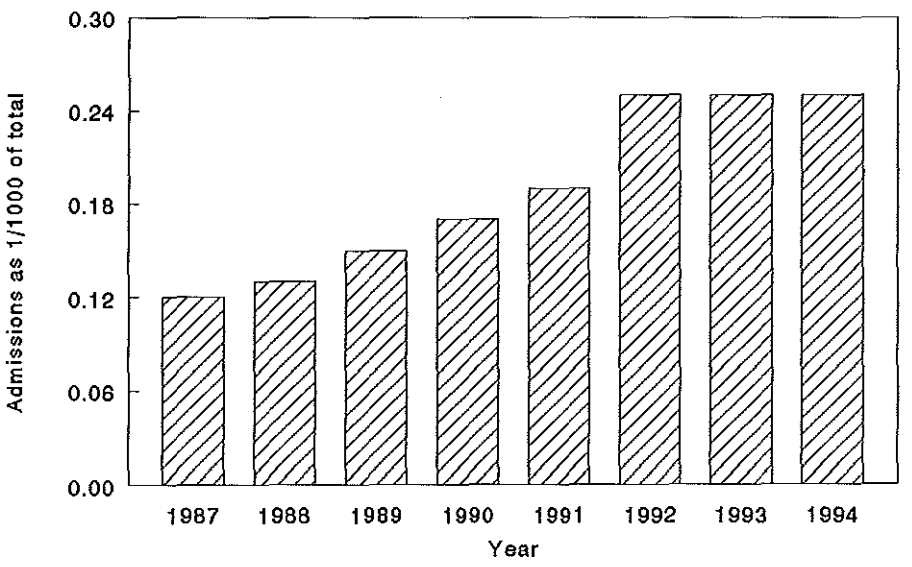
Discussion

This study was performed in order to examine the drug-related causes of hospital admissions because of agranulocytosis in The Netherlands, with a population based case-cohort design.

Although the misclassification might seem to be very high, excluded admissions mostly pertained to diagnoses closely related to agranulocytosis. These admissions were all excluded from our study, since it concerned most of the time pancytopenia, or a combination of agranulocytosis with anemia or thrombocytopenia, and the responsible drugs were not dispensed by a community pharmacy. Obviously, the classification of agranulocytosis of the Dutch Centre for Health Care Information contained a large number of admissions which were not our topic of interest.

The number of hospitalisations in the Netherlands due to agranulocytosis has increased during the past years. In 1991, an increase was noted in the number of admissions to Dutch hospitals because of agranulocytosis. Whereas in 1987 there were 182/1,502,018 admissions because of agranulocytosis, this number more than doubled to 393/1,582,458 in 1994 (Figure 3). Based on our study,

Figure 3
Admissions due to agranulocytosis



no specific product could be held responsible for this surge in admissions. However, the number of patients admitted because of agranulocytosis due to chemotherapy has risen throughout these years, indicating that these are probably the responsible agents.

In our study, many data were required for the clinical validation of agranulocytosis (as e.g. laboratory and bone marrow results). Although in 82% of admissions a response was received, not all included the information required for classification. Moreover, only admissions of patients with community acquired agranulocytosis could be used for the relative risk estimations, as in-hospital exposure data were not available. Since there appeared to be a large group of patients who developed agranulocytosis due to chemotherapy, only a small group of patients was left for analysis.

In this study, we were not able to assess an incidence rate of leucopenia as not all patients will have been admitted. It is likely, however, that few symptomatic cases will have been missed and that our study gives a fairly accurate estimation of the cumulative incidence of symptomatic agranulocytosis in the community outside the hospital. It should be noted that patients could have been admitted with agranulocytosis and coded otherwise in the registry of the Dutch Centre for Health Care Information. In order to assess false-negative misclassification, we added three diagnosis codes which could have included cases of agranulocytosis, and found only one possible case. It is still possible, however, that cases of agranulocytosis were coded otherwise, although this seems to be unlikely. Thyreostatic drugs had the highest relative risk and excess risk of drug-associated agranulocytosis, but also co-trimoxazole, sulphasalazine, clomipramine and dipyrone combined with analgesics were associated with high risk estimates.

Obviously, the cumulative incidence of agranulocytosis is much higher during admission in the hospital, especially in those specialized on cancer treatment, but in the absence of good drug exposure data concerning inpatients this cumulative incidence is difficult to estimate. We were interested, however, in the increase in admissions because of agranulocytosis to causes encountered outside the hospital. Although the PHARMO RLS exposure data are a sample of the drug use in the Dutch population, these data are good estimators of drug exposure [23]. Another reason for choosing the PHARMO RLS system as a source of exposure data is that these dispensing data are the only available data on an individual patient level in the period 1987 - 1990.

For this study we considered several types of epidemiologic designs. Because of the low incidence of agranulocytosis, we did not consider a cohort study as a useful approach. Case-control studies are very suitable for studying rare diseases. We had several reasons for not using a case-control design. First, recall bias would have been introduced into the study. As agranulocytosis is an impressive type of event that patients are not likely to forget, it would not have

been easy to find controls subject to the same recall of exposure as cases. Second, as drugs are a well-known cause of agranulocytosis, physicians might inquire more insistently about drug use in the index than in the control group. Third, although agranulocytosis is considered to be a rare disease, the low population exposure prevalence of some drugs (e.g. thyreostatics) could consequently have meant that none of the controls would have been exposed to those drugs. Therefore, we used a case-cohort design, in which the cases were all patients admitted because of agranulocytosis in The Netherlands in the period 1987 to 1990 and the control cohort was a reference cohort of the Dutch population.

Theoretically, our analysis could have been adversely influenced by two issues. First, bias or confounding might have played a role. Selection bias might occur if agranulocytosis to one drug is more severe than agranulocytosis to another, or if patients with agranulocytosis to a particular drug are admitted more readily than patients with agranulocytosis to another drug. However, there are no reasons to believe that agranulocytosis to orally administered thiamazole or sulphasalazine has a worse prognosis than agranulocytosis to other orally administered drugs. Hence, this will mean that the proportion of community acquired cases of agranulocytosis which leads to admission is more or less the same for these drugs. Information bias might result if doctors who anticipate an increased risk of agranulocytosis perform more blood tests. This could occur, for instance, in patients on thyreostatic agents as these are a well-known cause of agranulocytosis. As all cases were symptomatic, however, this is not a likely explanation because symptomatic agranulocytosis will almost always lead to admission. Information bias by differential recall of drug use by patients ('recall bias') was not a problem as the information came from automated pharmacies and had been gathered before disease onset. In those patients in whom drug use could be checked in pharmacy data or general practitioner's records, 85% of drugs mentioned in the hospital data could be confirmed. Although it was possible to obtain the filling data on most cases, it was virtually impossible to get these data over a longer episode than the risk period. Therefore, dose and duration related risk estimates could not be obtained. Confounding is unlikely, as apart from drugs there are few independent risk factors for agranulocytosis, and we adjusted for age, gender, and concurrent drug use.

The second issue is that the reference cohort was not a random sample from the total study base. It is logistically impossible to take a random sample from the total study base of all inhabitants in The Netherlands. Instead of this we used a representative sample of community pharmacies, located in different parts of the country to minimize the role of regional differences in prescribing by medical practitioners. Thus, we do not believe that the exposure in the reference cohort differs substantially from the exposure in the total study base,

especially not as the vital statistics concerning age and gender of the reference cohort were the same as that of the population in The Netherlands [23].

In the IAAAS the overall incidence of community acquired agranulocytosis was estimated at 3.4 per million inhabitants per year [24], which is slightly higher than the 1.6 to 2.5 per million inhabitants per year found in our study. The IAAAS has been heavily debated, since bias was thought to play a role [15 - 18]. One of the difficulties was, that the rate ratio regarding dipyrone varied between regions from 0.8 to 23.7 [4]. The researchers could not explain this result, and thought it could be related to the differences in use of dipyrone in these regions: the risks were high in regions where the drug was not used very often, and low in those regions where use was common. Insofar as we are aware of, our study is the first which includes *all* cases of admitted agranulocytosis from a whole country. Our results were comparable to the IAAAS with regards to the elevated risks found for thyreostatic drugs and dipyrone, although the absolute number of cases to dipyrone was small. For thyreostatics a relative risk of 102 was found in the IAAAS (excess risk 6.3 per million users during one week of exposure) [5], which is comparable to the relative risk of 115 (excess risk 4.9 per million users during 10 days of exposure) found in the current study. There were not enough data to compare the different thyreostatics, but from the figures in the IAAAS paper one can deduct that also in this study the risk for thiamazole seems to be higher than for carbimazole [5]. Since carbimazole is converted to thiamazole in vivo, this higher risk is difficult to explain. With regards to anti-infective agents, an elevated relative risk was found for co-trimoxazole (12, excess risk 1.6 per million with two weeks exposure) and the macrolides (excess risk 7.1 per million) [6]. Cardiovascular drugs found to have an elevated relative risk were propranolol (relative risk 2.5, excess risk 0.1 per million with one week exposure), digoxin and acetyldigoxin (relative risk 2.5 and 9.9 respectively, excess risk 0.1 and 0.3) [8].

Two main types of mechanisms of drug-induced agranulocytosis have been recognized: the first is a dose-related toxic reaction (e.g. phenothiazines, antithyroid drugs, and chloramphenicol), and the second is an immune or allergic reaction (e.g. dipyrone). For most drugs it is unclear by which mechanism agranulocytosis is induced. Several drugs found in this study to have an elevated relative risk have been associated with agranulocytosis in the medical literature, including diuretics (e.g. chlorthalidone), antithyroid drugs (carbimazole, thiamazole, and propylthiouracil), penicillins, indomethacin, acetaminophen, dipyrone, benzodiazepines, antidepressants (e.g. amitriptyline), sulphasalazine, co-trimoxazole, carbamazepine, and phenothiazines [1]. For several other drugs, e.g. coumarins, digoxin, and prednisone this was not the case, although in the IAAAS also an elevated relative risk was found for digoxin and prednisone, for which the authors had no explanation. As in our

patients agranulocytosis disappeared despite continuation of these drugs, the association with these two drugs is probably not causal.

In conclusion, we found a slightly lower cumulative yearly incidence of community-acquired agranulocytosis in The Netherlands than was found in the IAAAS. In our study, thyreostatic drugs, co-trimoxazole, sulphasalazine, clomipramine and dipyrone combined with analgesics were associated with the highest risks of agranulocytosis.

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Part V

General discussion and summary

10

General discussion

The nationwide, population based case-cohort design

This thesis concerns the quantitative aspects of adverse drug reaction issues encountered at the Drug Safety Unit of the Inspectorate for Health Care in The Netherlands (DSU), and focuses on two serious adverse events which are frequently associated with drugs, namely anaphylaxis and agranulocytosis. The data from the DSU were not suitable for estimating incidences or relative risks of adverse events and their suspected causes. Therefore we looked for ways to investigate these rare adverse events. The main problem was, that not only were the adverse events rare, also the suspected drugs were not widely used. Cohort studies are suitable for studying rare exposure, but not rare outcome, and case-control studies are suitable for studying rare outcome, but not rare exposure. In this discussion, we will elaborate on our choice for a nationwide, population based case-cohort design.

The theoretical considerations concerning the nationwide, population based case-cohort design have been discussed in chapter 7. Why did we choose this design for the studies on anaphylaxis and agranulocytosis? In other words, what are the advantages and limitations of this approach? It is important to emphasize that although there are clear theoretical differences between a cohort, case-control and case-cohort design, all can be used to estimate relative risks. Even though a case-control study is more vulnerable to bias than a prospective cohort study and is (unless it is population based) less suitable for incidence assessment of disease, all designs may give valid risk estimates. Population based cohort studies on a nationwide scale have been performed in The Netherlands when studying rare exposure such as ibopamine [1] and acitretine [2]. On a similar scale, population based case-control studies have been performed on rare diseases such as Guillain-Barré syndrome [3] and primary pulmonary hypertension [4]. Here, the discussion focuses on the comparison between the current population based case-cohort design, and population based cohort and case-control designs when studying rare drug-associated events to rarely used drugs.

Obviously, a cohort study was not feasible when studying these rare events because this would require such a large cohort that the cost would be enormous. Hence, the most reasonable choice was between a population based case-cohort design, and a study with a population based case-control design with the same sample of cases.

Ideally, every epidemiological study is *valid, precise* and *efficient*. In other words: the risk estimates should be correct, the confidence intervals narrow (indicating that when the study is repeated, the risk estimates largely remain the same), and the study should not be unduly expensive or of long duration. Also important is the external validity, or *generalisability* of the study. Below, we will discuss the

value and shortcomings on each of these items of the case-cohort and case-control designs respectively. The results of this discussion are summarised in Table 1.

The *validity* of a study may be endangered by *chance*, *bias* and *confounding*. Although chance findings can never be excluded, the possibility that a significantly increased risk estimate is explained solely by chance is reduced to 5% by convention and use of statistical techniques. The aspect of chance will not be dicussed in our comparison of the case-control and case-cohort design.

Bias is usually divided into *selection bias* (or *sample distortion bias*), and *information bias*. *Selection bias* is an error due to systematic differences in characteristics between those who are selected for the study and those who are not [5]. If identification of study subjects takes place on the basis of outcome, this occurs only when these systematic differences are related to the exposure of interest. In the current examples, both a case-cohort and a case-control design would have required the same sample of cases, obtained via all general and university hospitals in The Netherlands. In both case-cohort studies in this thesis, most clinical data were obtained anonymously, without identifying patients or physicians, or sometimes even hospitals. In a case-control study, however, we would have had to contact the hospital and the physician to obtain controls from the same hospital or outpatient setting. This could have resulted in a much larger non-response than the approximately 15% non-response which we encountered in the case-cohort studies. Moreover, the lower response rate in a case-control study could have been biased, as in the study of anaphylaxis, for instance, the role of glafenine was a well-known topic. A selective response in cases of glafenine-associated anaphylaxis could have inflated the relative risk estimations. Apart from selective response in obtaining clinical data from cases, the choice of a control group would have been a problem. It is very difficult to choose a consistent hospital control group of persons who do not have a higher or lower chance of using the drugs of interest. Ideally, one should choose community controls, but this would further have jeopardized the response rate, as it would have required sequential permission of hospitals, internists, general practitioners and patients. In the case-cohort design, however, community controls were included in the reference cohort.

Also, selection bias could have occurred if physicians admitted patients because they were aware of the fact that their patients had used the exposure of interest ("referral bias") [6]. In both study types, there would have been a similar possibility that referral bias had played a role, as both would have used the same sample of admitted cases. Such selection bias was, however, unlikely. In case of anaphylaxis, a general practitioner will refer a patient on the basis of severity, irrespective of the cause. Also agranulocytosis is a serious event, warranting admission to a hospital. In both study types, patients who were treated at home or died at home did not enter the case group. We thought it unlikely that many

patients would be treated at home for such serious events as anaphylaxis or agranulocytosis, and as distances are small in The Netherlands it seemed unlikely that patients would have died at home from these illnesses without having been admitted to a hospital.

In the case of agranulocytosis, when regular blood counts are performed because this is advised in the product information of a drug, this could lead to "detection bias". For this reason, we restricted our analysis to symptomatic cases, thereby excluding "cases" which were the result of such routine assessments. This feature would be a problem in both the case-control and the case-cohort design. Although there is essentially no difference in the potential bias in the selection of cases for both study designs, the selection of controls in a case-control design may be biased, whereas this is not a problem in the case-cohort design as described in this thesis.

Nondifferential misclassification of disease in the data from the Dutch Centre for Health Care Information could have occurred [7]. In order to account for positive misclassification, validation of the admissions took place. We tried to account for negative misclassification by including several admission diagnoses which could have included cases of anaphylaxis or agranulocytosis, and did not find many misclassified admissions. Bias among the admissions selected for the study might have taken place if patients with an adverse reaction to one particular drug were coded as the adverse reaction, and patients with the same adverse reaction to another drug were coded differently, e.g. agranulocytosis as leukopenia, and anaphylaxis as urticaria. We did not find evidence to support this.

Information bias is a flaw in measuring exposure or outcome that results in differential quality (accuracy) of information between compared groups [5]. *Information bias* might occur, for instance, when patients with a disease remembered more about their drug use than healthy controls ("recall bias") [6]. In the case-cohort design, recall bias was not a problem as the exposure histories of the control group were gathered from the sample of pharmacies before disease onset. Although this was not a random sample of pharmacies, several comparisons showed us that the drug exposure data were representative of drug use in The Netherlands. In order to account for differences in prescription behaviour, data from several parts of The Netherlands were included. Since all pharmacies in the six PHARMO cities are covered by the database, changing from one pharmacy to another does not affect the data in the database. In a case-control study, however, such data would not have been available, as pharmacy data on many of the cases and controls would no longer be available, due to the time lag between occurrence and registration in the data base of the Dutch Centre for Health Care Information. Both from the point of view of the vulnerability to *selection bias* and *information bias*, the case-cohort has advantages over the case-control design when considering the current topics of interest.

Confounding occurs when another factor than the exposure of interest is an independent risk factor for the outcome of interest and also associated with the exposure of interest, without being an intermediate step in the causal pathway [6,7]. Here, a case-control may have advantages over a case-cohort design, as data on the presence or absence of all potential risk factors can be obtained quite easily. In the case-cohort design, information about such other risk factors are usually not available on all members of the reference cohort. In the current examples, i.e. anaphylaxis and agranulocytosis, this was not a problem of importance. First, because both diseases are mostly caused by drugs and have few other independent risk factors. Second, because these other known independent risk factors are not associated with the drugs that cause anaphylaxis and agranulocytosis respectively. On the other hand, unless data on other risk factors are gathered from the reference cohort, this means that the value of the current nationwide population based case-cohort design is limited to diseases with a high drug-attributed etiological fraction.

Table 1. Advantages and limitations of case-control and case-cohort designs.

	Case-control	Case-cohort
Suitable for studying rare disease	+	+
Suitable for studying rare exposure	-	+
Can examine multiple exposure	+	+
Probability of high response rate	+/-	+
Controls can be used for several studies	+/-	+
Subject to recall bias	+	-
Subject to selection bias	+	+/-
Co-factor/confounder data available	+	+/-
Efficient	+	++
Well suited for evaluation of disease with long latent period	+	+
Incidence rates computable if population based	+	+
Generalisability	+	+

The *precision* of a study is reflected in the 95%-confidence intervals. The precision may be endangered by too few cases or controls. As we were not able to increase the number of well-documented (admitted) cases of both rare events, we needed a large reference group. In the study on anaphylaxis, for instance, we

would have had a fair chance that none of the controls in a case-control study would have been exposed to glafenine, as its use had strongly declined. For this reason, a case-cohort study had obvious advantages.

The *efficiency* of an epidemiological study tells us how fast, and at what price a study of sufficient validity and precision can be performed. From the point of view of efficiency, the case-cohort study was both faster and cheaper than a case-control study. It would have cost time and money to obtain permission for the study from hospitals, physicians, and patients, and drug exposure data from the control group itself in a case-control study. We were, however, able to work with anonymized clinical data on the cases alone in the case-cohort study, and had exposure data of the reference cohort already available.

The *generalisability* (external validity) of a study does not so much depend on whether the sample is representative of the total population, but more on whether the results can be extrapolated to the total population [7]. As we included all admitted symptomatic cases in The Netherlands, this applies to both the anaphylaxis and agranulocytosis study, irrespective of the question as to whether we would have chosen a case-control or a case-cohort design.

Conclusion

Both the validity, precision and efficiency of the case-cohort design were sufficient for studying the outcomes of interest. This type of design may be very useful when there is a need to study serious but rare diseases to rarely used drugs, especially when rapid results are required, as is often the case when problems arise shortly after the introduction of new pharmacological entities. There are, however, also limitations to this approach. The main limitation is that, except for age and gender, no data are available on non-drug-related risk factors. This limitation can be dealt with, however, by obtaining such data at regular intervals from random samples from the reference cohort.

Also, it is only possible to investigate adverse reactions, which are so serious that they will lead to hospital admission, since hospital admission data are used. It is not always possible to estimate the incidence of adverse reactions, since those patients that have not been admitted to the hospital (but were treated at home or died at home) are not included in the study, and all adverse reactions which were not coded as a principal diagnosis, but as an additional diagnosis (mostly adverse reactions due to drugs that were given in the hospital) are not included as well. However, when dealing with an adverse reaction which is so serious that it will always lead to hospital admission, one can assume that no cases have been missed, and in this situation it is possible to estimate the incidence.

In conclusion, the case-cohort design has proved to be a useful design for studying rare events with a high attributable risk of drugs, such as anaphylaxis or agranulocytosis.

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summary

Adverse drug reaction monitoring started to develop in the nineteen-fifties, but it was strongly stimulated by the thalidomide disaster in 1961. Until recently, adverse drug reaction monitoring has been performed by the national reporting centres in different countries, which also pool summarised data at the World Health Organisation Collaborating Centre for International Drug Monitoring in Uppsala. Most important data, however, used to come from the medical literature, but some centres made a substantial contribution to the detection of unknown adverse reactions. Pharmaceutical firms have their own adverse reaction registration systems. Several problems have been encountered with adverse drug reaction monitoring systems, the main problem being selective recognition and reporting of suspected adverse reactions. Another major problem is, that sales figures on drugs are not always available. In clinical trials, the most frequent adverse events are usually detected, but rare adverse events are mostly not discovered, since only small numbers of patients are involved. Also, the elderly, children and pregnant women, use of co-medication, co-existence of other diseases, and longterm use are mostly not examined in clinical trials. The increasing availability of computers and large databases has made it possible, not only to monitor data on large numbers of patients, but also to process these data fairly easily. This has stimulated the rapid advance of epidemiology, and recently the development of pharmacoepidemiology as a subspeciality, encountering several specific problems when studying drug use, adverse reactions to drugs, and other drug effects. In chapter 1, the problems in assessing drug safety, and the systems and methods for pharmacoepidemiology are discussed.

The scope of this thesis was to examine the usefulness of the nationwide population based case-cohort design when studying rare, but serious, adverse events to rarely used and/or newly marketed drugs. Therefore, attention was focused on two adverse reactions which are both rare and often caused by drugs, i.e. anaphylaxis and agranulocytosis (chapter 2).

The studies in this thesis were started because of a problem encountered at the Drug Safety Unit of the Inspectorate for Health Care in The Netherlands, formerly the Netherlands Centre for Monitoring of Adverse Reactions to Drugs. Since 1974, a large number of reports on glafenine-associated anaphylaxis were received, reaching a peak in 1979. The problem was that it was impossible to prove solely on the basis of reporting figures, that the number of glafenine-associated anaphylactic reactions was indeed higher than the number of anaphylactic reactions associated with other analgesics. Due to the publicity on glafenine and its adverse events, it was conceivable that glafenine-associated adverse events were reported relatively more often than adverse events due to other drugs. This was also emphasised by the marketing authorization holder, who maintained that the large number of reports was based solely on reporting bias. Therefore, a study was performed on all reports on drug-associated anaphylaxis made in the past 20 years to the Drug Safety Unit. This study not only showed

that slightly more than one third of all reports on anaphylaxis were associated with glafenine, but also, that there was a large number of reports on trimethoprim-associated anaphylaxis, which have been described separately as a case series (chapter 3). The study on all reports of drug-associated anaphylaxis is described in chapter 5. Since this study gave rise to alarming figures on glafenine-associated anaphylaxis, a suitable study design was sought to cope with the problem of reporting bias.

Another problem at the Drug Safety Unit with a rare, but serious, adverse event pertained to drug-associated agranulocytosis, and mainly agranulocytosis due to antidepressants. A case of agranulocytosis associated with trazodone was reported, and is described in chapter 4. There were also reports and studies in the literature, suggesting that mianserin caused agranulocytosis fairly often, but the number of epidemiological studies substantiating this were scanty. In the eighties, a large case-control study had been performed, the International Agranulocytosis and Aplastic Anemia Study, but in this period mianserin had not yet been widely marketed. Therefore, we started with a review of all reports on drug-associated agranulocytosis (chapter 6). This showed that next to dipyrone, mianserin was most often reported as a suspected cause of drug-induced agranulocytosis. Again the problem of reporting bias was thought to play a role, and a suitable study design for this problem was needed as well.

The main problem encountered was not only that anaphylaxis and agranulocytosis are rare diseases. In the case of glafenine the sales figures had declined dramatically, and in the case of mianserin the drug, being an antidepressant, had not been sold on such a large scale. Thus, the exposure to these drugs was rare as well. Cohort studies are suitable for studying rare exposure, but not rare disease, and in general case-control studies are suitable for studying rare disease, but not rare exposure. Kupper et al., Miettinen and Prentice had published about a case-cohort study as an efficient design to reduce the number of subjects for whom covariate data are required. This is especially efficient if the processing of raw materials takes a lot of time. In this situation, for different reasons, the case-cohort design seemed to be an efficient method to study drug-associated anaphylaxis and agranulocytosis. A data base concerning data on morbidity was already available (the Dutch Centre for Health Care Information), as was a database concerning data on exposure in a sample of the Dutch population (the PHARMO RLS system). A nationwide population based case-cohort design was therefore used. The theoretical considerations concerning this design are described in chapter 7. In short, the exposure in people with the disease ("cases") is compared to the exposure in a reference cohort taken from the total Dutch population.

The study on drug-associated anaphylaxis performed with this case-cohort design is described in chapter 8. An elevated relative risk was not only found for glafenine, but also for amoxycillin, penicillins in general, and the group of

analgesic drugs. The risk of anaphylaxis to glafenine relative to analgesics, however, was still significantly elevated. This study was mainly responsible for the withdrawal of glafenine from the market in a large number of countries in 1992.

Next, a case-cohort study on drug-associated agranulocytosis was performed. This study did not show a large relative risk for mianserin, but it did show an elevated risk for thyreostatics, sulphasalazine, and non-steroidal anti-inflammatory drugs (chapter 9). The results from this study were mainly similar to those found in the International Agranulocytosis and Aplastic Anemia Study, but higher relative risks and risk differences were found for thyreostatic agents and dipyrone.

In the final discussion (chapter 10), the advantages and limitations are discussed of the nationwide population based case-cohort design for pharmacoepidemiology. Advantages are that there is no recall bias, less risk of selection bias than in a case-control study, and that the study design is suitable for studying rarely used drugs. The main limitation is that, with the exception of age and gender, there are no data on other risk factors than drug use. A solution would be to collect such data at regular intervals in samples from the reference cohort. Another limitation is the fact that it is only possible to estimate the incidence of adverse reactions that are so serious that these will always lead to hospital admission.

It is concluded that the nationwide population based case-cohort design is relatively quick and cost-effective when assessing the drug-associated risks of rare but serious adverse events with a high drug-attributed etiological fraction.

Samenvatting

Het systematisch registreren van bijwerkingen van geneesmiddelen begon in de jaren '50, en werd sterk gestimuleerd door de thalidomide (Softenon®) affaire in 1961. Tot op heden wordt de registratie van gemelde bijwerkingen in diverse landen gehouden door de nationale overheden, die tevens een samenvatting van hun data gezamenlijk onderbrengen bij het "Collaborating Centre for International Drug Monitoring" van de Wereld Gezondheids Organisatie in Uppsala. De belangrijkste gegevens over bijwerkingen van geneesmiddelen komen echter nog steeds uit de medische literatuur, hoewel enkele centra een substantiële bijdrage leverden aan het ontdekken van onbekende bijwerkingen. Farmaceutische bedrijven hebben hun eigen registratiesysteem voor bijwerkingen. Registratiesystemen voor bijwerkingen hebben diverse tekortkomingen, met name onderrapportage, selectieve herkenning en melding van effecten die ten onrechte als bijwerking werden gekenschetst. Een ander groot probleem is dat betrouwbare gegevens over het daadwerkelijk gebruik van geneesmiddelen doorgaans ontbreken. In klinisch onderzoek voorafgaand aan registratie worden de frequentst voorkomende bijwerkingen meestal ontdekt, maar zeldzame bijwerkingen niet. Dit komt door de beperkte omvang van klinisch onderzoek. Bovendien worden ouderen, kinderen en zwangere vrouwen doorgaans van dit onderzoek uitgesloten, en wordt de rol van comedicatie, nevenaandoeningen en langdurig gebruik meestal niet onderzocht in klinisch onderzoek. De toenemende beschikbaarheid van computers en grote databestanden heeft het mogelijk gemaakt om niet alleen grote aantallen patiënten te volgen, maar ook om deze data vrij gemakkelijk te verwerken. Deze ontwikkeling is een belangrijke stimulans voor farmaco-epidemiologisch onderzoek gebleken. In de farmaco-epidemiologie doen zich enkele problemen voor, die kenmerkend zijn voor het bestuderen van geneesmiddelengebruik, bijwerkingen van geneesmiddelen, en andere effecten van geneesmiddelen. In hoofdstuk 1 zijn de problemen besproken die zich voordoen bij het vaststellen van de veiligheid van geneesmiddelen, en de systemen en methoden voor farmaco-epidemiologisch onderzoek.

Het doel van dit proefschrift was de bruikbaarheid te onderzoeken van een landelijke case-cohort studie opzet bij het bestuderen van zeldzame, maar ernstige bijwerkingen van geneesmiddelen die weinig gebruikt worden en/of recent op de markt zijn gekomen. Hiertoe werd de aandacht gericht op twee bijwerkingen die beiden zeldzaam zijn en vaak veroorzaakt worden door geneesmiddelen, namelijk anafylaxie en agranulocytose (hoofdstuk 2).

De studies in dit proefschrift werden opgezet vanwege een probleem waarmee het Bureau Bijwerkingen Geneesmiddelen van de Inspectie voor de Gezondheidszorg geconfronteerd werd. Sinds 1974 werd een groot aantal meldingen ontvangen van anafylactische reacties toegeschreven aan het gebruik van glafenine, waarbij een piek was bereikt in 1979. Het probleem was dat het onmogelijk was om alleen op basis van het aantal meldingen aan te tonen, dat de frequentie van anafylactische reakties door glafenine inderdaad hoger was dan het

aantal anafylactische reakties door andere analgetica. Door de publiciteit over de bijwerkingen van glafenine bestond de mogelijkheid dat bijwerkingen toegeschreven aan glafenine relatief vaker gemeld zouden worden dan bijwerkingen toegeschreven aan andere geneesmiddelen. Dit werd ook door de fabrikant benadrukt, die van mening was dat het grote aantal meldingen gebaseerd was op selectief melden. Derhalve werd een studie verricht naar alle bij de Inspectie voor de Gezondheidszorg gemelde gevallen van anafylaxie door geneesmiddelen in de afgelopen 20 jaar. Deze studie liet niet alleen zien dat iets meer dan één derde van alle meldingen betreffende anafylaxie toegeschreven waren aan glafenine, maar ook dat er een groot aantal meldingen waren over anafylaxie toegeschreven aan trimethoprim, welke laatste serie ziektegeschiedenissen apart zijn beschreven in hoofdstuk 3. De studie over alle meldingen van anafylaxie toegeschreven aan geneesmiddelen is beschreven in hoofdstuk 5. Gezien de omvang van het aantal meldingen van anafylaxie toegeschreven aan glafenine, werd een geschikte studie opzet gezocht om het probleem van het selectieve melden te ondervangen.

Een ander probleem op het gebied van zeldzame, maar ernstige bijwerkingen betrof agranulocytose door antidepressiva. Een voorbeeld hiervan was een melding van agranulocytose toegeschreven aan trazodone (hoofdstuk 4). Er waren ook meldingen en studies in de literatuur die suggereerden dat mianserine vrij vaak agranulocytose veroorzaakt, maar er waren weinig epidemiologische studies die dit konden onderbouwen. In de jaren '80 was een grote case-control studie verricht, de Internationale Agranulocytose en Aplastische Anemie Studie, maar in deze periode werd mianserine nog betrekkelijk beperkt gebruikt. Eerst werd een overzicht gemaakt van alle meldingen van agranulocytose toegeschreven aan geneesmiddelen (hoofdstuk 6). Afgaand op de meldingen werden dipyrone en mianserine het vaakst als verdachte oorzaak van agranulocytose door geneesmiddelen benoemd. Opnieuw echter leek het probleem van selectief melden een rol te spelen, en een geschikte studie opzet voor dit probleem was daarom ook belangrijk.

Het probleem was niet alleen dat anafylaxie en agranulocytose zeldzame ziekten zijn. In het geval van glafenine waren de verkoopcijfers sterk afgenomen, terwijl mianserine, een antidepressivum, op betrekkelijk beperkte schaal werd toegepast. Derhalve was het gedeelte van de bevolking dat deze geneesmiddelen gebruikte ook klein. Cohort studies zijn geschikt voor het bestuderen van zeldzaam toegepaste geneesmiddelen (expositie), maar niet van zeldzame ziekten, en in het algemeen zijn case-control studies geschikt voor het bestuderen van zeldzame ziekten, maar niet van zeldzaam toegepaste geneesmiddelen. Kupper et al., Miettinen en Prentice hadden in de literatuur gesuggereerd dat een case-cohort studie opzet een efficiënte aanpak vormt wanneer het nodig is het aantal patiënten waarover gegevens nodig zijn te beperken. Dit is met name efficiënt als het verkrijgen van aanvullende gegevens veel tijd kost. Om diverse redenen leek in

deze situatie de case-cohort studie opzet een efficiënte methode om anafylaxie en agranulocytose door geneesmiddelen te bestuderen. Een gegevensbestand met betrekking tot morbiditeit was al beschikbaar (SIG Zorginformatie), evenals een gegevensbestand met betrekking tot gegevens over expositie in een steekproef van de Nederlandse bevolking (het PHARMO RLS systeem). Derhalve werd een landelijke case-cohort studie opzet gekozen, waarin de gehele bevolking als cohort beschouwd werd. De theoretische overwegingen met betrekking tot deze studie opzet zijn beschreven in hoofdstuk 7. In het kort komt dit erop neer dat de expositie bij personen met anafylaxie of agranulocytose ("cases") vergeleken werd met de expositie in een referentie cohort uit de totale Nederlandse bevolking.

De studie over anafylaxie die is uitgevoerd met deze case-cohort studie opzet wordt beschreven in hoofdstuk 8. Een verhoogd relatief risico werd niet alleen gevonden voor glafenine, maar ook voor amoxicilline, penicillines in het algemeen, en de groep van analgetica. Het risico op anafylaxie door glafenine was echter significant verhoogd ten opzichte van analgetica. Deze studie vormde de belangrijkste aanleiding voor het terugtrekken van glafenine van de markt in een groot aantal landen in 1992.

Vervolgens werd een case-cohort studie naar agranulocytose uitgevoerd. Deze studie liet geen hoog relatief risico zien voor mianserine, maar wel voor thyreostatica, sulfasalazine, en niet-steroïde anti-inflammatoire geneesmiddelen (NSAID's) (hoofdstuk 9). De resultaten van deze studie waren in grote lijnen gelijk aan die van de Internationale Agranulocytose en Aplastische Anemie Studie, maar er werden hogere relatieve risico's en risico verschillen gevonden voor thyreostatica en dipyrone.

In de uiteindelijke discussie (hoofdstuk 10), zijn de voor- en nadelen besproken van de landelijke case-cohort studie opzet in de farmaco-epidemiologie. Voordelen zijn dat er geen sprake is van recall bias, dat er minder kans is op selection bias dan bij een case-control studie, en het feit dat deze studie opzet geschikt is voor zeldzaam gebruikte geneesmiddelen. De belangrijkste beperking is dat, met uitzondering van geslacht en leeftijd, geen gegevens beschikbaar zijn met betrekking tot andere risicofactoren dan geneesmiddelgebruik. Een oplossing hiervoor zou zijn om zulke gegevens met regelmatige intervallen te verzamelen in steekproeven van het referentiecohort. Een andere beperking vormt het feit dat het alleen mogelijk is een incidentie te schatten van bijwerkingen die zo ernstig zijn, dat ze bijna altijd tot ziekenhuisopname zullen leiden.

De conclusie is, dat de landelijke case-cohort studie een relatief snelle en kosten-effectieve studie opzet is om aan geneesmiddelen toegeschreven zeldzame bijwerkingen te kwantificeren.

Dankwoord

Velen hebben een bijdrage geleverd aan de totstandkoming van dit proefschrift. Hen wil ik hier graag bedanken.

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Curriculum vitae

Melanie Marguerite van der Klauw was born on November 30, 1965, in Enschede. From 1978 to 1984 she followed Gymnasium ß at the Gymnasium Erasmianum in Rotterdam. She started her study in Medicine at the Erasmus University in Rotterdam in the same year, and finished her Propaedeuse in 1985 cum laude. She obtained her doctoral degree in September 1988 and her medical degree in November 1990.
During her study, from 1986 to 1988, she worked as a student-assistent with Prof. dr. A. Versprille at the Pathofysiological Laboratory of the department of Pulmonary Diseases of the Erasmus University Rotterdam.
Also during her study, she started with the work that has led to this thesis at the Department of Internal Medicine II (Prof. J.H.P. Wilson). She worked as a research fellow at his department from December 1990 until October 1994. In between, from May to August 1992 she worked as a resident (AGNIO) at the department of Internal Medicine II at the University Hospital Dijkzigt, Rotterdam (Prof. J.H.P. Wilson). From April 1993 to October 1994 she worked parttime at the Netherlands Centre for Monitoring of Adverse Reactions to Drugs in Rijswijk, and parttime as a research fellow at the Department of Internal Medicine II. In May and June 1993 she took a Summercourse in Epidemiology and Biostatistics at McGill University in Montreal, Canada.
In October 1994 she started with her training in internal medicine at the Drechtsteden Hospital, location Refaja, in Dordrecht (Dr. B.P. Hazenberg, Dr. A.C.M. van Vliet), which she will continue at the Department of Internal Medicine II at the University Hospital Dijkzigt (Prof. J.H.P. Wilson).

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